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STUDIES ON VARIATION IN PATHOGENICITY IN LEAF RUST OF WHEAT, *PUCCINIA TRITICINA* ERIKSS.[1]

BY A. M. BROWN AND T. JOHNSON[2]

Abstract

In experiments designed to clarify the causes of the pathogenic changes that have recently been encountered in leaf rust of wheat, two factors were given consideration, (1) the possibility that nuclear exchanges might occur between the mycelia of different races in the uredial stage, and (2) the possible role of *Thalictrum* species in originating new rust strains. Infection studies with mixtures of races 9 and 15 and mixtures of races 5 and 9 did not produce any evidence that these races could interchange nuclei and thus initiate new pathogenic strains of leaf rust.

In greenhouse infection tests with native *Thalictrum* spp., aecia were produced on *T. dasycarpum*, pycnia only on *T. dioicum* and *T. polygamum*, while no infection of *T. venulosum* took place. In similar tests with the introduced species *T. glaucum* and *T. dipterocarpum*, abundant production of aecia occurred on both species. In out-of-doors inoculation tests with *T. dasycarpum* and *T. venulosum*, no infection of these two species took place, whereas, under the same conditions, heavy aecial production occurred on the introduced species *T. glaucum*.

Selfing studies in which *Thalictrum glaucum* was infected with known physiologic races have indicated that some races of leaf rust are homozygous, and others heterozygous for pathogenic characters. A culture of race 5 appeared to be homozygous, whereas a culture of race 3 was heterozygous, giving rise to races 3, 15, 32, 68, and three undescribed races. A culture of race 76 was heterozygous for both pathogenicity and urediospore color. Aeciospores of this race produced uredia of two different shades of yellow in addition to uredia of normal color. Aeciospores derived from teliospores collected in the field also gave rise to uredial strains of yellow spore color. Most of the yellow rust strains were decidedly low in pathogenic vigor.

Introduction

Leaf rust of wheat (*Puccinia triticina* Erikss. = *P. rubigo-vera Tritici* (Erikss. & Henn.) Carl.) is one of the most highly specialized of the cereal rusts. In the third (1942) revision of the "International Register" of physiologic races of this rust, 129 races are described of which 68 have been encountered in North America. In recent years it has become evident that the physiologic race population in North America is by no means stable. It has been found (14) that certain wheat varieties that formerly showed considerable resistance to leaf rust in the field have become year by year more

[1] *Manuscript received June 2, 1949.*

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susceptible. This increase in susceptibility has been attributed to the widespread occurrence of race 128 and new biotypes of races 5 and 15. Other races that have been regarded (16) as important in this respect are 44, 58, and 126.

The question of the possible explanations of these pathogenic changes in leaf rust is one of obvious importance both practically and theoretically: practically, because an understanding of the process might suggest control measures; theoretically, because of the natural human interest in the mechanism of evolutionary processes.

On the basis of present knowledge, there appear to be three possible explanations for these pathogenic changes: (1) the function of alternate hosts, (2) the role of nuclear interchange in the uredial stage of the rust, (3) mutation. The work reported in the present paper was undertaken principally with the idea of exploring the possible function of the alternate host (*Thalictrum* spp.) and the role of nuclear interchange in the uredial stage as causes of variation in the leaf rust organism.

The Possible Role of Nuclear Interchange in the Uredial Stage

It has been generally assumed that nuclear interchange in the uredial stage is not a factor in variation in the cereal rusts. This assumption is based on the knowledge that, in the uredial stage, the rust is in a dikaryotic condition in which the two associated nuclei divide conjugately and thereby supposedly remain in constant association with each other. The process of conjugate division is, therefore, thought to confer on the rust a stability somewhat comparable to that prevailing in plants with a diploid nucleus.

The experience of rust investigators appears to bear out this hypothesis. In *Puccinia graminis Tritici* Erikss. & Henn., physiologic races are frequently kept in pure culture for many years without any variant arising from them. Stakman *et al.* (21) refer to a uredial culture of race 1 of this rust that remained "constant pathogenically for more than 13 years". Evidence appears also to be lacking that physiologic races of this rust interchange nuclei in the uredial stage. Watson (22), in a study of competition between races 17, 19, 34, 56, and 147, does not report that he isolated from the mixed cultures any other races than those that entered into them. In nature, under epiphytotic conditions, there must occur between mycelia innumerable contacts that might furnish opportunities for nuclear exchange within a physiologic race or between physiologic races. Nevertheless, experience has shown that in the rust area of North America certain physiologic races of wheat stem rust, such as 56, 17, 38, and 32, recur year after year in much the same proportions. If nuclear exchange between the races of this rust were at all common, a much greater variety of races would be expected.

In *P. triticina*, it has been suggested by Ruth Allen (1), on the basis of cytological studies, that fusions may take place between two appressoria or two substomatal vesicles, which would obviously afford opportunities for

nuclear interchange between different mycelia. In a recent study, Rodenhiser and Hurd-Karrer (20) observed "fusion bodies" formed through anastomoses between germ tubes of the urediospores of wheat leaf rust germinating on nutrient agar, and noted a similar phenomenon on the surface of inoculated wheat leaves.

The above observations, though suggestive, do not prove that two or more mycelia of a rust can interchange nuclei. The only proof known to the writers that a dikaryotic rust mycelium can transmit a nucleus to another mycelium rests on the work of Brown (4, 5) who showed that dikaryotic mycelia of *Puccinia Helianthi* Schw., *Uromyces Fabae* (Pers.) de Bary, and *U. trifolii-hybridi* (W. H. Davis) Arth. were capable of dikaryotizing the haploid mycelia of their respective rusts.

If a dikaryotic mycelium can transmit a nucleus to a haploid (monokaryotic) mycelium, it would seem possible that two dikaryotic mycelia might interchange nuclei.

In an attempt to determine to what extent nuclear interchange occurred when dikaryotic mycelia of known races of leaf rust came into contact, uredio-spore inoculum composed of a mixture of two readily distinguishable races was increased by infecting a susceptible wheat (Little Club). The uredio-spores of the next generation were employed for the inoculation of the standard differential leaf rust hosts. In this manner, race 9 was mixed with race 15 and, separately, with a biotype of race 5, designated as race 5*a*. The cultures of each race originated from a single spore.

To ensure that the mycelia of the paired races would have ample opportunity of coming into contact, all inoculations with the mixed inocula were carried out by the use of an abundant spore supply and, in general, infections were closely spaced on the leaves. When the infection results on the differential hosts were recorded, particular care was taken to look for types of infection not characteristic of either of the paired races, e.g., type 3 or type 4 infections on the varieties Carina and Brevit.

The results of these tests were entirely negative—visible evidences of racial interactions were lacking and only the races entering into a mixture were reisolated from it. Fifteen reisolates from the pairings of races 9 and 15 were identified as either race 9 or race 15; and six reisolates from the pairings of races 9 and 5*a* were identified as one or the other of these two races. Despite the undoubted occurrence of numerous opportunities for the interchange of nuclei between the paired races, there is no evidence that such interchanges did occur.

These negative results do not prove that physiologic races of *P. triticina* are incapable of interchanging nuclei in the uredial stage. As nothing appears to be known about the effect on pathogenicity of such nuclear exchange, if it does occur, it is just possible that the nuclei in interlacing mycelia were reassociated without any visible pathogenic effect. It seems, however, reasonably safe to conclude that, as far as the races studied are concerned, nuclear interchange in the uredial stage is not of frequent occurrence.

Races Originating from *Thalictrum* spp. Infected with Field Collections of Teliospores

As the sexual stage in the life cycle of leaf rust occurs on *Thalictrum* spp., it was thought that a study of the rust derived from that host might give some indication of the range of variability in leaf rust. Accordingly, two collections of teliospores gathered in the fall of 1946 at Winnipeg, Man., and Kapuskasing, Ont., were induced to germinate by subjecting them to periods of alternate wetting and drying during November. This material had not been subjected to freezing except possibly light frosts prior to the collection of the spores late in September. The teliospores collected at Winnipeg on Sept. 28 began to germinate about the middle of December and continued to do so until late in the following April.*

Sporidia from the Winnipeg teliospores were sown on young plants of *Thalictrum glaucum* Desf.,** which had proved to be a congenial host. Aeciospores derived from this host gave rise to 22 uredial isolates among which there were present the following races (the number of isolates of each race in brackets): race 1 (4); race 5 (1); race 15 (4); race 29 (2); race 76 (1); race 89 (3); race 113 (1); race 113*a*† (2); race 128*a* (4). The infection types produced by these races are shown in Table I.

Sporidia from the Kapuskasing teliospores were sown on *Thalictrum dipterocarpum* Franch. on which numerous aecia were produced. These gave rise to 14 isolates comprising the following races: race 1 (1); race 5 (1); race 15 (6); race 30 (1); race 37 (1); race 39 (1); race 126 (1); and two new races designated as C-1†† (one isolate) and C-2 (one isolate).

In these isolates of aecial origin there were present six races not found in the field in 1946 (the summer in which the above-mentioned teliospores were formed), namely races 30, 37, 39, 89, C-1, and C-2. On the other hand, certain races common in field collections were not represented in the isolates from aecia, such as races 3, 5*a*, 9, 15*a*, and 58, or were scantily represented, such as race 76.

In the rust of aecial origin, variation of a kind never encountered in uredial collections was observed, such as strains of red urediospore color, to which the wheat variety Little Club was resistant, and strains of yellow urediospore color. The latter occurred only in the rust derived from the teliospores collected at Kapuskasing, Ont. Four yellowish cultures, two of them bright yellow and two orange-yellow, were isolated. All were so pathogenically

** The ability of leaf rust teliospores to germinate without undergoing a rest period at low temperature may not be unusual, for teliospores of races 1a, 5a, and 35, collected from greenhouse cultures on June 24, 1948, were induced to germinate a week later. They caused the infection of young Thalictrum plants and aecia were obtained in July.*

*** The writers are indebted to Prof. C. W. Lowe, Department of Botany, University of Manitoba, for assistance with the identification of this host.*

† Races designated by the letter "a" are virulent towards Hope, H-44, and the various wheats derived from them.

†† Isolates that did not closely approximate any of the 129 physiologic races described in the "International Register" were designated as C-1, C-2, etc., the letter C representing Canada. The infection types of these isolates are recorded in Tables I and II.

TABLE I

INFECTION TYPES PRODUCED ON DIFFERENTIAL HOSTS BY PHYSIOLOGIC RACES OF LEAF RUST, *Puccinia triticina*, ISOLATED FROM AECIAL CULTURES THAT ORIGINATED FROM TELIOSPORES COLLECTED AT WINNIPEG, MAN., AND KAPUSKASING, ONT. (ANY CULTURE THAT DEVIATED ONLY SLIGHTLY FROM SOME KNOWN RACE WAS ASCRIBED TO THE RACE IT RESEMBLED MOST CLOSELY)

Races isolated		Differential hosts									No. of times identified
		Norka	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Hope	
From teliospores collected at Winnipeg	1	0	0–1	1	0–1	0–1	1	1+	1	x	4
	5	3	0–1	0–1	0–1	0–1	3	1	3	x	1
	15	0–1	0–1	1–2	1	1–2	3	2+	3	x	4
	29	3	2–3	2–3	x	3+	1	3	1	2–3	2
	76	0	x	3	x	3+	3+	2	3	x	1
	89	3+	2–3	2–3	2–3	3+	3+	3	3+	x	3
	113	3	2–3	2–3	2–3	2–3	3	2+	3	x	1
	113*a**	3	2–3	2–3	2–3	2–3	3	2+	3	3	2
	128*a*	3	1–2	2–3	x	2–3	1	3	1	3	4
From teliospores collected at Kapuskasing	1	0	0	0–1	0	0–1	0–1	1	1	x	1
	5	3	0–1	0–1	0–1	0	3+	1+	3+	x	1
	15	0	0	0–1	0–1	0–1	3	1+	3	x	6
	30	3+	2–3	2–3	3–	3	3+	1–2	3+	x	1
	37	3+	2+	2+	2+	3	1–2	2+	1	x	1
	39	3+	2–3	2–3	0–1	3	3	1–2	3+	x	1
	126	3	1	1	0–1	3	3+	1	3+	x	1
	C-1	0	1	1	3	3	2	1	2	x	1
	C-2	0	2	2	3	3	3	1	3	x	1

Definition of infection types in terms of host reaction:
0 = immunity; 1 = resistance; 2 = moderate resistance;
3 = moderate susceptibility; 3+ = susceptibility;
x = indeterminate reaction.

** Cultures designated by the letter "a" are virulent to the variety Hope.*

weak on Little Club and other wheats that they were lost before any congenial host could be found. Several isolates of normal color were also pathogenically weak on Little Club and were lost for that reason.

Selfing Studies with Known Races

In *P. graminis Tritici* (12), and in *Melampsora Lini* (9), it has been shown that in most cases the inheritance of pathogenic characteristics proceeds in accordance with Mendelian laws. In crosses between races, certain infection types on a given host are dominant, others are recessive. In the selfing of a race of *P. graminis Tritici* (that is, its passage through the barberry and a study of the infection types in the next uredial generation), the race will

show segregation for dominant pathogenic traits in a heterozygous condition but not for recessive traits.

As nothing was known about the inheritance of the pathogenicity of leaf rust races, it was thought worthwhile to carry out selfing studies with some of the races in the hope of discovering the mode of inheritance of their pathogenic characteristics (infection types) on the differential hosts.

Several races of leaf rust, originating from monosporous cultures, produced teliospores in June, 1947. These teliospores were stored in a refrigerator at a temperature just above freezing until October, when they were taken out and alternately wetted and dried at room temperature. Early in November the teliospores began to germinate and were used to inoculate seedling plants of *T. glaucum*. Good pycnia of physiologic races 3 and 5*a* were obtained in December and these races were individually selfed by intermixing the nectar of their pycnia.

As the aecia that subsequently formed did not develop protruding cups, single-cup isolations were impractical but single-pustule isolations were readily obtained and the aeciospores were sown on seedling plants of Little Club. When the uredia developed, single-pustule isolates were made, increased, and cultured on sets of differential hosts.

Selfing of Race 3

Sixteen isolates of race 3 were studied, of which 11 were assigned to races already described in the International Register, and five were identified as one or the other of three new races. The reactions of the differential hosts to these races together with the times each race was isolated are shown in Table II.

A notable feature of the seven races isolated in this study was their exact resemblance to the parent race in their inability to rust the variety Norka and in the production of an x type of infection on the variety Hope. On Loros, Mediterranean, and Democrat, the type 3 infection of the parent race recurred in the progeny except for race 15, which produced a type 1 infection on Loros and race 68 which produced a type 1 on Mediterranean and Democrat. On Hussar there was also one deviation from the type 1 infection produced by the parent race, namely, the type 3 infection produced by race C-3. Infection types on Carina, Brevit, and Webster, however, showed considerable variation that is largely responsible for the variety of physiologic races derived from this selfing study.

From these results it may be concluded that the factors governing the infection capabilities of race 3 on Norka and Hope are in a homozygous condition, while those governing its infection capabilities on the other differential hosts are in a heterozygous condition.

Selfing of Race 5a

The results secured in the selfing of race 5*a*, as shown in Table II, indicate the possibility that the factors governing its infection capabilities on all the differential hosts are present in a homozygous condition. In this study, 29

TABLE II

INFECTION TYPES PRODUCED ON DIFFERENTIAL HOSTS BY PHYSIOLOGIC RACES OF WHEAT LEAF RUST, *P. triticina*, ISOLATED FROM THE SELFING OF RACES 3, 5*a*, AND 76. (ANY CULTURE THAT DEVIATED ONLY SLIGHTLY FROM SOME KNOWN RACE WAS ASCRIBED TO THE RACE IT RESEMBLED MOST CLOSELY)

Physiologic races isolated	Differential hosts									No. of times identified
	Norka	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Hope	
From race 3 selfed										
3	0	1	2	1–2	3	3	1	3	x	4
15	0	0–1	1	0–1	1	3+	1–2	3	x	4
32	0	2	2+	2+	3	3	1	3	x	1
68	0	3	3	3	3	1	1	1	x	2
C 2	0	2+	2+	3	3+	3+	1	3+	x	1
C-3	0	2+	2+	2+	3	3	3	3	x	3
C-4	0	2–3	2–3	3	3	2–3	1	2–3	x	1
From race 5*a* selfed										
5*a*	3+	0	0–1	0–1	0–1	3+	1–2	3+	3	27
68	0	3	3+	3	3	1+	1+	1	x	1
105	3	0–1	3–	0–1	3	3	1	3	x	1
From race 76 selfed*										
1*a*	0	0	0	0	0	1	1	0	3	2
29*a*	3	2–3	3–	2–3–	3–	0–1	3	0–1	3–	2
101*a*	3	x	x	2	3	x	3	x	3	1
Light yellow 1	1	0–1	1+	0	0–1	0	1	1	1	5
Mars yellow 10*a*	3	3	3	3	3	0–1	0–1	0–1	3	3

* *For types of infection produced by race 76 see Table* 1.

single pustule (uredial) isolates were identified as follows: 27 were assigned to race 5*a*; one to race 105, which is separated from the parent race by the susceptible reactions of Brevit and Loros and the indeterminate reaction of the variety Hope; and one to race 68, which is distinguished from the parent race by the reactions of all the hosts except Hussar. Norka, Mediterranean, and Democrat are all susceptible to race 5*a* but resistant to race 68. Hope is susceptible to the former race, but indeterminate in reaction to the latter. On the other hand, Carina, Brevit, Webster, and Loros are resistant to race 5*a* but susceptible to race 68.

This complete reversal in pathogenicity is difficult to explain and it may have been due to race admixture in the parent culture, in which case selfing might have been supplanted unwittingly by crossing, or by the selfing of an admixed race.

Selfing of Race 76

During March and April, 1948, pycnial cultures of race 76 were selfed and isolates originating from aecia were cultured and observed in a manner similar

to that just described. Altogether 13 uredial isolates were derived from the selfed cultures. Of these, eight developed uredia of yellowish color while the remaining five produced uredia of normal color. The cultures developing yellow uredia were subsequently segregated into two color groups, a light yellow and a deeper yellow—approximately the "Mars Yellow" of Ridgway (18). The light yellow cultures were all identified as race 1, while the Mars yellow cultures, all of them alike in pathogenicity, were identified as race 10.

From a study of the 13 isolates derived from the selfing of race 76, it may be seen (Table II) that the inability of race 76 to attack Norka was expressed in seven isolates, the uredia of two being normal in color while those of the other five were light yellow. The remaining six isolates attacked Norka heavily. The pathogenicity on Mediterranean and Democrat of all 13 isolates differed strikingly from that of the parent race. These varieties, susceptible to the parent race, were resistant to all 13 isolates. On the remaining differential hosts, Carina, Brevit, Webster, Loros, and Hussar, there was considerable variation in pathogenicity among the different isolates. Perhaps the most striking result encountered was the difference in pathogenicity of the two yellow rusts. The isolates that produced light-yellow uredia, and were identified as race 1, attacked weakly not only the differential hosts, but also Little Club, and consequently were maintained with difficulty. On the other hand, the isolates with deeper-colored uredia (Mars Yellow), although not as virulent as the isolates with normal colored uredia, nevertheless attacked heavily Norka, Carina, Brevit, Webster, Loros, and Hope.

Although the number of isolates observed was not large, the results obtained suggest that race 76 was heterozygous for pathogenicity and also for urediospore color.

Reaction of *Thalictrum* Species and Their Possible Role in Nature

Despite the fact that several species of the genus *Thalictrum* have been proved to be more or less susceptible to *P. triticina* in greenhouse tests (8, 11, 17), the opinion held by most investigators is that *Thalictrum* species do not function actively in nature as alternate hosts of this rust (see Chester (7, p. 44); Johnson and Newton (13, p. 351)).

It is clear that, in North America, there is no such definite relationship between *Thalictrum* and wheat leaf rust as there is between *Berberis* and wheat stem rust. *Thalictrum* species produce no visible spread of leaf rust to adjacent fields of wheat. It is, nevertheless, possible that wheat leaf rust may occasionally produce aecia on one or another of the native species of *Thalictrum* and that the aeciospores may, in turn, cause infection on wheat. Such occasional infections would have little or no significance from the point of view of leaf rust epidemiology but might be an important factor in the origination of new leaf rust races. At all events, this aspect of the leaf rust problem should not be left out of consideration, particularly in view of the opinion expressed by Arthur (2, p. 181) that aecia of wheat leaf rust undoubtedly do occur on

Thalictrum species in North America although no collections of aecia have as yet been identified with that rust.

The determination of the true role of *Thalictrum* spp. in this connection is rendered more difficult by their susceptibility to several other rusts of the complex species *Puccinia rubigo-vera* (DC.) Wint. Cultural studies are therefore necessary before any given aecial infection can definitely be related to its graminaceous host.

In the Prairie Provinces of Canada, aecia do not occur abundantly on *Thalictrum* species, and the scanty evidence available indicates that such aecia as do occur belong to the *agropyrina* and *Agropyri* varieties of *P. rubigo-vera.* Fraser and Conners (10) attribute collections of aecia on *T. dasycarpum* Fisch. & Lall. and *T. venulosum* Trel. to *P. Clematidis* (DC.) Lagerh. = *P. Agropyri* Ell. & Ev. = *P. rubigo-vera Agropyri* (Erikss.) Arth. Bisby *et al.* (3) agree with this conclusion but with the reservation that the rust in Manitoba may possibly in some cases belong to var. *agropyrina.* One of the writers (T.J.) made two collections of aecia on *T. venulosum* at Winnipeg, Man., in 1935, that according to Arthur's Manual (2) were attributable to var. *agropyrina.* Neither collection would infect wheat. Aeciospores collected on this host at Clear Lake, Man., in 1947 likewise failed to infect wheat and were also attributed to variety *agropyrina.*

In 1948, attempts were made to determine the reaction to wheat leaf rust of several *Thalictrum* species both in the greenhouse and out-of-doors. In the greenhouse, germinating teliospores of several physiologic races were suspended over young plants of the native American species *T. dasycarpum, T. dioicum,* T. venulosum,** and *T. polygamum* Muhl.* and the two introduced species *T. dipterocarpum* and *T. glaucum.* Of these, the last two proved to be congenial hosts on which abundant aecia were formed. Of the native species, pycnia and aecia developed moderately well on *T. dasycarpum* and aeciospores taken from this host infected Little Club wheat. This species, however, appeared to be a less congenial host than the two introduced species. A weak development of pycnia was observed on *T. dioicum* and *T. polygamum,* but intermixing of the scanty nectar did not lead to aecial production. No infection occurred on *T. venulosum.*

Out-of-doors, in May, 1948, straws bearing viable teliospores of leaf rust of wheat were strewn in woodlots where the two native species *T. dasycarpum* and *T. venulosum* were plentiful, and in a sheltered garden where the introduced species *T. glaucum* had been planted. Weather was generally favorable for teliospore germination, and, on June 12, numerous aecia were observed on the plants of *T. glaucum.* The aeciospores from these were sown on Little Club wheat, which became infected. Aecia were again observed on the same plants

* *These species of Thalictrum were received through the co-operation of Dr. H. A. Senn, Ottawa.*

on July 12 and again the aeciospores infected Little Club wheat. Throughout this period, the native species, *T. dasycarpum* and *T. venulosum*, remained free from infection.

These experiments indicate that the four native species tested (*T. dasycarpum*, *dioicum*, *polygamum*, and *venulosum*) are definitely less congenial hosts of wheat leaf rust than are the two introduced species *T. glaucum* and *T. dipterocarpum*. Though not clearly proved by the experiments, it seems probable that all the species are more resistant when grown out-of-doors than in the greenhouse. In view of the fact that *T. dasycarpum* became infected and produced aecia in the greenhouse, there is some possibility that this species might bear aecia out-of-doors. Its failure to become infected in the out-of-doors trial mentioned above does not prove that it cannot become infected.

It appears to the writers that the status of native *Thalictrum* species with regard to leaf rust of wheat is by no means settled. It seems particularly necessary to study further the reaction to leaf rust of *T. dasycarpum*, *T. dioicum*, and *T. polygamum*.

Discussion

The most important problem confronting investigators of the cereal rusts is probably that of determining the causes and mechanism of variation, particularly variation in pathogenic potentialities. In the case of any rust that possesses an alternate host, it is generally assumed that variation occurs during the sojourn of the rust on that host. In assessing the situation with regard to leaf rust of wheat, one is confronted with the fact that it is not known with certainty whether or not an alternate host functions in the propagation of the rust. It is reasonably certain that an alternate host does not cause any widespread dissemination of this rust. It is not at all certain that an occasional aecial pustule on an alternate host may not occur; and, if it occurs, there exists a possibility that it may give rise to a new physiologic race of the rust.

Although species of the genus *Thalictrum* are generally regarded as the alternate hosts of leaf rust, it may well be that certain species of other ranunculaceous genera are equally functional. It has been shown (6) that, in Siberia, *Isopyrum fumarioides* bears aecia of wheat leaf rust. The related plant *I. biternatum* occurs in North America, but, as Mains (17) was not able to infect this species in greenhouse tests, there is no reason to suppose that it functions as an alternate host. Nevertheless, further studies should be carried out on the reaction to leaf rust of this and related plants, including species of genera known to be hosts of the varieties *Agropyri* or *agropyrina* of *P. rubigo-vera*.

If it could be proved definitely that the aecial hosts of *P. triticina* do not function as alternate hosts under natural conditions, there remains only the possibility that variation in this rust takes place in the uredial stage. It

seems not at all unlikely that a rust that does not utilize an alternate host might develop an alternative mechanism of variation. In this connection two possible mechanisms come to mind, i.e. (1) interchange of nuclei between different clones and (2) mutation.

The failure to secure evidence, in the present paper, for interchange of nuclei between races of leaf rust should not be regarded as definitive evidence against the operation of that process. As for mutation, no active investigation has been undertaken but, in view of certain reports (15, 19) of mutation in this rust, it is probable that mutation occurring in the uredial stage is one of the sources of variation. If any adequate understanding of the origin of variation in leaf rust is to be achieved, it is essential that further study be given to both the above mentioned processes.

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INHERITANCE OF PATHOGENICITY AND UREDIOSPORE COLOR IN CROSSES BETWEEN PHYSIOLOGIC RACES OF OAT STEM RUST[1]

By T. Johnson[2]

Abstract

In the F_1 generation of crosses between physiologic races 7 (orange) and 11 (red) of *Puccinia graminis* Pers. var. *Avenae* Erikss. and Henn., the medium sized uredia (type 2 infection) formed by race 11 on the oat variety White Tartar were dominant to the large (type 4) uredia of race 7, and the small (type 1) uredia of race 7 on the variety Richland were dominant to the type 4 uredia formed by race 11. On the varieties Sevnothree and Joanette Strain, the F_1 hybrids produced the same type of infection as did the "maternal" parent race, that is, hybrids from race 7 × race 11 crosses produced a type 4 infection like race 7, and reciprocal hybrids produced a type 1 infection like race 11. These facts led to the suggestion that the cytoplasm of the maternal parent race influenced the infection type of the F_1 hybrid on these two oat varieties.

A study of the F_2 generation of the cross race 11 × race 7 showed that on the varieties White Tartar and Richland the dominant and recessive infection types appeared in a ratio of 9 : 7, which suggests that their inheritance is governed by two pairs of complementary genes. The distribution of physiologic races in F_2 conforms to this assumption and indicates that the genes governing infection types on these two varieties associate at random to produce physiologic races 1, 11, 3, and 4. These races occurred in F_2 in a ratio of 31 : 20 : 22 : 12, as compared to an expected ratio of 27 : 21 : 21 : 16. On the variety Sevnothree, 84 of 85 F_2 cultures produced type 1 uredia, in this way resembling the maternal parent, race 1, and the maternal grandparent, race 11. One F_2 culture, only, produced the type 4 uredia characteristic of the paternal grandparent, race 7. It is concluded from this study, and from crosses between races 1 and 2, that the maternal (cytoplasmic) influence evidenced in the F_1 generation persists in F_2 and F_3.

In F_1, the red urediospore color of race 11 was dominant to the orange color of race 7. The distribution in these two color classes in F_2 and F_3 suggests that the inheritance of urediospore color is governed by a single pair of genes.

In crosses between physiologic races of *Puccinia graminis* Pers. var. *Tritici* Erikss. & Henn., studies have been made of the inheritance of pathogenicity and urediospore color in the F_1, F_2, and F_3 generations of certain crosses (2, 4, 5, 6, 7). No comparable studies have, as yet, been recorded for *P. graminis* Pers. var. *Avenae* Erikss. & Henn., although crossing results, confined to the F_1 generation, have been reported (3). As some information is now available on the inheritance, in the F_2 and F_3 generations, of urediospore color and of certain pathogenic characteristics, it is the purpose of the present paper to present these results and, as far as possible, to interpret them.

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A Cross Between Race 7 and Race 11

F_1 Generation

The cross under consideration was one between race 7 and race 11—formerly described as race 10*a* (3). The infection types produced by the two races are illustrated diagrammatically in Fig. 1. In addition to differences in pathogenicity, the two races differed in the color of the urediospores. Race 11 produced uredia of the usual red color characteristic of oat stem rust, whereas race 7 produced uredia of a bright orange color. Before the cross was made, both parental races had been subjected to selfing studies from which it was known that race 11 was homozygous for color of uredia and for the infection types produced on the differential varieties White Tartar, Richland, and Joanette Strain, whereas race 7 was homozygous for color of uredia and for infection types on White Tartar and Joanette Strain but heterozygous for its infection type on Richland. The progeny of race 7 consisted predominantly of race 7, which produces small round uredia on Richland, but there were present also a number of cultures of race 6, which produces large linear uredia on this variety.

TABLE I

INFECTION TYPES OF RACES 7 AND 11 OF *P. graminis* VAR. *Avenae* AND OF THE F_1 PROGENY OF CROSSES BETWEEN THESE TWO RACES

	White Tartar	Richland	Joanette Strain and Sevnothree
Parent race 7	(4)	(1)	(4)
Parent race 11	(2)	(4)	(1)
F_1, 7 × 11 (7 crosses)	(2)	(1)	(4) = race 2 red
(9 crosses)	(2)	(4)	(4) = race 8 red
F_1, 11 × 7 (6 crosses)	(2)	(1)	(1) = race 1 red
(4 crosses)	(2)	(4)	(1) = race 11 red

A total of 26 crosses were made between the two races (Table I). In the 16 crosses, race 7 orange × race 11 red, pycniospores were transferred from haploid pustules of race 11 to haploid pustules of race 7, and the hybrid aeciospores therefore arose from haploid mycelia of race 7 that had received pycniospore nuclei of race 11. Of these 16 crosses, seven produced race 2 red and nine produced race 8 red. Of the 10 crosses in which pycniospores were transferred in the reverse direction, six produced race 1 red and four produced race 11 red. The occurrence of two races in each case was due to the heterozygosity of race 7 orange for infection type on Richland.

In these crosses, red urediospore color was dominant to orange; the medium sized uredia (type 2) produced by race 11 on White Tartar were dominant to the large uredia (type 4) produced by race 7; and the small uredia produced by race 7 on Richland (type 1) were dominant to the large ones produced by

race 11. In each cross, the infection type on the variety Joanette Strain resembled that of the maternal parent race, i.e., the race from whose haploid mycelia the aecia were formed. The inheritance of the infection types expressed on this variety is further discussed elsewhere in this paper.

Progeny Studies in F_2 and F_3

Only one of the 26 F_1 hybrids obtained in the above-mentioned crosses was submitted to further study. This hybrid, a culture of race 1 from the cross shown in Fig. 1, produced teliospores that germinated and caused infection on several barberry plants. The study of the distribution of rust character-

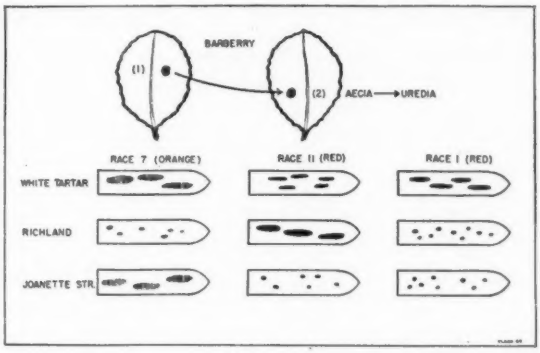

FIG. 1. *Diagram illustrating the cross race 11 (red) × race 7 (orange). Above—barberry leaves, as drawn when the cross was made, showing that nectar was transferred from one haploid pustule of race 7 to a haploid pustule of race 11. The hybrid aeciospores formed in pustule (2) gave rise to the F_1 uredial generation, race 1 (red). Below—diagrammatic representation of infection types produced by the parent races and the hybrid race on the three differential oat varieties.*

istics in the F_2 generation was based on cultures established from 81 single aecia picked off at random from four barberry plants. From these aecia, 81 cultures of monoaecial origin were established. Of these, 77 produced a single physiologic race in each culture, whereas four produced two races in each. Thus the F_2 generation studied comprised a total of 85 isolates.

The distribution of the physiologic races present in these isolates was as follows:

Race 1—31 isolates (23 red; 8 orange)
Race 3—22 isolates (13 red; 9 orange)
Race 4—12 isolates (8 red; 4 orange)
Race 8— 1 isolate (orange)
Race 11—19 isolates (11 red; 8 orange)

Subsequently, three of the F_2 isolates were selected for selfing studies designed to elucidate the inheritance of rust characters in the F_3 generation, namely, one isolate of race 3 red, one of race 4 orange, and one of race 8 orange.

Each of these isolates was brought into the telial stage and the teliospores were, in due course, induced to germinate and infect barberry. All produced normal pycnia and aecia on the barberry. As in F_2, the F_3 studies were based on aecia selected at random. The results of the F_2 and the F_3 studies are shown in diagrammatic form in Fig. 2.

The Inheritance of Urediospore Color in F_2 and F_3

The data available on the distribution of urediospore color in the F_2 and the F_3 generation are summarized in Table II.

TABLE II

Actual and theoretical distributions of red and orange uredial cultures in F_2 and F_3 and goodness of fit to a 3 : 1 ratio

	Actual distribution	Theoretical distribution	
F_2 generation			
(1)*Red	55	63.75	$\chi^2 = 4.804$
Orange	30	21.25	$P = 0.05 - 0.02$
(2) Red	217	202.5	$\chi^2 = 4.153$
Orange	53	67.5	$P = 0.05 - 0.02$
F_3 generation			
(3) Red	53	51	$\chi^2 = 0.313$
Orange	15	17	$P = 0.7 - 0.5$

*(*1*) = *Data from 85 cultures derived from aecia selected at random (see Fig. 2).*

(*2*) = *Data from counts of red and orange pustules on oat leaves inoculated with mass transfers of aecia.*

(*3*) = *Data from selfing of an F_2 culture of race 3 red (see Fig. 2).*

Two sets of data were obtained, one from cultures derived from the random selection of aecia mentioned above, the other from a count of red and orange uredia on oat seedlings inoculated by mass collections of aeciospores. It may be noted here that the two sets of data bearing on color distribution in F_2 are not in close agreement. The data derived from the 85 uredial cultures originating from a random collection of aecia show a ratio of 1.8 red to 1 orange, whereas data derived from a count of uredia on oat leaves inoculated with a mass collection of aecia show a ratio of 4.1 to 1.

The F_3 data are limited to the selfing of one F_2 culture, race 3 red, from which 68 aecia selected at random gave rise to as many uredial cultures. The ratio in this group of cultures was 3.5 red to 1 orange, a reasonably good fit to a 3 : 1 ratio.

It seems most probable from a consideration of the available data that the inheritance of urediospore color is governed by a single pair of genes that produce a 3 : 1 ratio in the selfing of any heterozygous culture. As both red and orange spores contain the yellow or orange cytoplasmic pigment, the

only difference between the two is that the red spores contain in the spore wall a pigment that is virtually absent from the orange spores. The pair of genes operative in the inheritance of color apparently governs the inheritance of this pigment.

Inheritance in F_2 and F_3 of Infection Types on White Tartar and Richland

The essential facts concerning the inheritance of pathogenicity in the F_2 and F_3 uredial generations are shown in Fig. 2. Only those infection types present in the two parent races occurred in F_2 and F_3; no blending or intermediacy was observed.

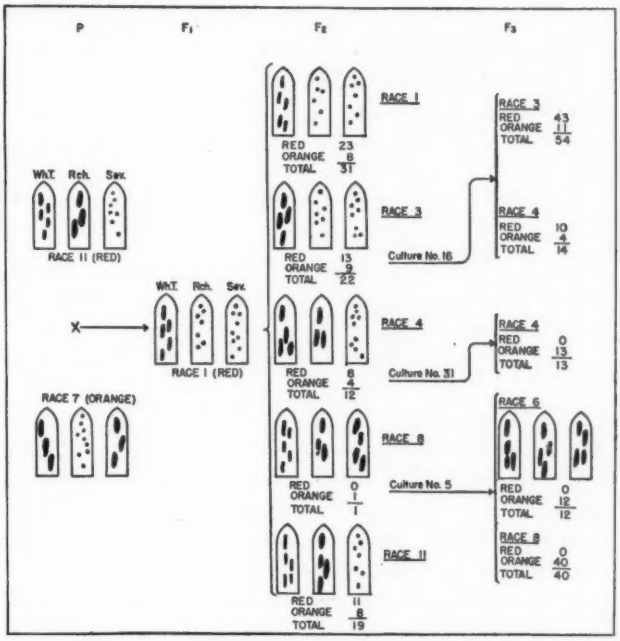

Fig. 2. *Distribution of physiologic races in F_2 and F_3 of the cross race 11 red × race 7 orange.*

On White Tartar and Richland the large, type 4 uredia, suppressed in F_1, reappeared in F_2. The infection types on these two hosts were associated in all possible combinations: type 2 on White Tartar with type 1 on Richland to produce race 1 (as in F_1). Type 4 on White Tartar with type 1 on Richland to produce race 3 (as in the race 7 parent). Type 2 on White Tartar with type 4 on Richland to produce races 8 and 11 (as in the race 11 parent). Type 4 on White Tartar with type 4 on Richland to produce race 4.

The last was the only combination not met with in the parents or the F_1 generation.

On the host variety Joanette Strain and on Sevnothree, a selection from it, there was no comparable segregation for infection type, the infection type being that of the race 11 parent except for the appearance of the 4 type in one culture (race 8). The inheritance of the infection types appearing on these varieties is discussed elsewhere in this paper.

The distribution of infection types on White Tartar and Richland in F_2 and F_3 may be used to arrive at a factorial basis for the inheritance of pathogenicity on these varieties. Table III shows that for both White Tartar and

TABLE III

ACTUAL AND THEORETICAL DISTRIBUTIONS IN F_2 OF TYPE 2 AND TYPE 4 INFECTIONS ON WHITE TARTAR, TYPE 1 AND TYPE 4 INFECTIONS ON RICHLAND, AND GOODNESS OF FIT OF THESE DISTRIBUTIONS TO A 9 : 7 RATIO

Infection type	Actual and theoretical distributions in F_2		
	Actual distribution	Theoretical distribution	
White Tartar			
(2)	51	47.8	$\chi^2 = 0.4895$
(4)	34	37.2	$P = 0.5 - 0.3$
Richland			
(1)	53	47.8	$\chi^2 = 1.2926$
(4)	32	37.2	$P = 0.3 - 0.2$

Richland the distribution of the contrasted infection types approximates a 9 : 7 ratio and therefore suggests that in each case the inheritance is governed by two pairs of genes that are complementary, the type 4 uredia appearing in the absence of the dominant genes of one pair or the other and in the absence of both pairs of dominant genes.

It may then be suggested that the genotypic constitutions of the parent races and the F_1 hybrid are as follows:

	White Tartar infection type	Genotype	Richland infection type	Genotype
Parent—race 11	(2)	AA BB	(4)	cc dd
Parent—race 7	(4)	aa bb	(1)	CC Dd or Cc DD
F_1 —race 1	(2)	Aa Bb	(1)	Cc Dd

There are two reasons for supposing that the genotype of the race 7 parent for infection type on Richland was CC Dd or Cc DD rather than Cc Dd. One is that the selfing of the parent culture produced a distribution of 36 cultures with type 1 infection as against only four cultures with type 4 infection—a ratio much closer to the 3 : 1 ratio expected from either of the first mentioned genotypes than to the 9 : 7 ratio expected from the genotype Cc Dd. The other reason is that in the 26 crosses between races 7 and 11 there occurred 13 F_1 hybrids with type 1 infection on Richland and 13 with type 4 infection, which is in exact agreement with expectation if the genotype of race 7 were CC Dd or Cc DD. To account for the 9 : 7 ratio of these infection types on Richland in F_2, it would have to be assumed that the F_1 culture, race 1, selected for selfing, possessed the genotype Cc Dd.

On the above assumptions the theoretical distribution of physiologic races in F_2 (taking into consideration only the infection types on White Tartar and Richland) should be as shown in Table IV, i.e., 27, 21, 21, and 16 for races 1, 11, 3, and 4, respectively. Statistical analysis indicates that the actual distribution, i.e., 31, 20, 22, and 12, is a reasonably good fit and may be interpreted as giving support to the genotypic constitutions shown above.

TABLE IV

ACTUAL AND THEORETICAL DISTRIBUTIONS OF PHYSIOLOGIC RACES IN F_2 BASED ON RUST BEHAVIOR ON THE VARIETIES WHITE TARTAR AND RICHLAND, THE THEORETICAL DISTRIBUTION BEING FOUNDED ON THE ASSUMPTION THAT TWO PAIRS OF GENES GOVERN THE EXPRESSION OF INFECTION TYPES ON EACH VARIETY

Infection types		Formulae*		Race	Actual distribution	Theoretical distribution	
White Tartar	Richland	White Tartar	Richland				
(2)	(1)	AB	CD	1	31	27	
(2)	(4)	AB	Cd	11 and 8	20	21	
		AB	cD				
		AB	cd				
(4)	(1)	Ab	CD	3	22	21	
		aB	CD				
		ab	CD				
(4)	(4)	Ab	Cd	4	12	16	$\chi^2 = 2.5450$
		Ab	cD				$P = 0.5\text{–}0.3$
		Ab	cd				
		aB	Cd				
		aB	cD				
		aB	cd				
		ab	Cd				
		ab	cD				
		ab	cd				

* *A given formula in some cases includes several genotypes. For example, formula Abcd includes genotypes AAbbccdd and Aabbccdd.*

The F_3 data obtained from the selfing of F_2 cultures of races 3 and 8 may be interpreted on the same assumption. Race 3 was homozygous for infection type on White Tartar but heterozygous for infection type on Richland, producing 54 cultures with type 1 (small) uredia on Richland and 14 cultures with type 4 (large) uredia. The actual ratio 54 : 14 is close enough to the theoretical ratio 51 : 17 to suggest a 3 : 1 ratio. On this supposition, the genotype governing the rust behavior of race 3 on Richland was either Cc DD or CC Dd. Race 8 was homozygous for infection type on Richland but heterozygous for that on White Tartar and produced an F_3 generation made up of 40 cultures with type 2 (medium size) uredia and 12 cultures with type 4 (large uredia), a distribution very close to the 39 : 13 distribution expected if the ratio were 3 : 1. A genotype of either Aa BB or AA Bb would account for the distribution of the two infection types on White Tartar.

The only other F_2 culture selfed, race 4, was, as would be expected, homozygous for the type 4 (large) uredia on both White Tartar and Richland.

It will be seen from Table IV that, whereas the original combinations of infection type 2 on White Tartar with type 4 on Richland that characterized the race 11 parent and type 4 on White Tartar with type 1 on Richland that characterized the race 7 parent recurred in F_2 with about the expected frequency, the new combinations, type 2 on White Tartar with type 1 on Richland and type 4 on White Tartar with type 4 on Richland, were respectively too high and too low. To test the possibility that these discrepancies might indicate association between type 2 on White Tartar and type 1 on Richland, the actual frequencies obtained for the different combinations, i.e., 31, 20, 22, and 12, were subjected to the test of independence as described by Fisher (1). The high P value of 0.8 to 0.7 indicated no association and would therefore suggest that no linkage exists between any of the characters.

The Inheritance of Infection Types on Sevnothree*

In considering the inheritance of the infection types of the parent races 7 orange and 11 red on the variety Sevnothree it is necessary to refer back to the results obtained in F_1 from the crosses as previously described (3). Both parent races were proved by selfing studies to be homozygous for the infection types on Joanette Strain and the selection Sevnothree, which was derived from it. Race 7 orange produced exclusively large (type 4) uredia and race 11 (then referred to as race 10*a*) exclusively small (type 1) uredia.

It will be seen from the data presented in Table I that the progeny arising from the crosses and reciprocal crosses differed only in one respect, namely, in the infection type on Sevnothree. In all cases in which the hybrid aeciospores were formed in haploid mycelia of race 7 orange, the hybrid rust produced on Sevnothree large (type 4) uredia that were identical with the uredia

** The variety Sevnothree, a selection from Joanette Strain, has replaced the latter as a differential host. Its reaction to the physiologic races discussed in this paper is identical with that of Joanette Strain. Any reference to infection types on one of these varieties applies equally to the other.*

produced by the race 7 parent rust except for their color, which was red instead of orange. In the reciprocal crosses in which the hybrid aeciospores arose from race 11 haplonts dikaryotized by race 7 nuclei, the hybrid rust produced small (type 1) uredia similar to those produced by the race 11 parent rust.

In reporting these crosses (3) it was stated that "this phenomenon does not seem to be capable of explanation on a chromosomal basis, but can be readily explained if it is assumed that the pycniospore nucleus of the paternal race reaches the protoaecium of the maternal race unaccompanied by its cytoplasm, which, in any case, is very limited on account of the minute size of the pycniospores. Each hybrid aeciospore would then receive from the paternal race only a nucleus but from the maternal race a nucleus plus cytoplasm."

This phenomenon was first encountered in crosses between physiologic races of *P. graminis* var. *Tritici* and was there expressed in the infection types produced on Marquis wheat (5, 6, 7). Progeny studies with hybrid rusts displaying this supposed cytoplasmic effect revealed that it persisted in a large measure in the F_2 generation (5, 6). Some variation, however, did exist in the infection types produced on Marquis by different F_2 rust lines, which indicated a possible segregation of nuclear factors affecting rust behavior on Marquis, a segregation, however, balanced or obscured by the cytoplasmic effect.

One of the reasons for the present study was to determine the expression in the F_2 generation of the supposed cytoplasmic effect observed in F_1 on Joanette Strain and Sevnothree. In the study of the 85 cultures comprising the F_2 generation, particular notice was taken of the infection type produced on the variety Sevnothree. As shown in Fig. 2, the F_2 cultures, with one exception, were classified as one or another of races 1, 3, 4, or 11, all of which produced a type 1 infection on Sevnothree. In the one exception just referred to, an F_2 culture with orange spores, large uredia were observed among the small uredia on Sevnothree. An isolation from the large uredia produced a pure culture of race 8 orange, which consistently produced large (type 4) uredia on Sevnothree, whereas an isolation from the small uredia gave rise to race 11 orange, pathogenically identical with the other culture except that it produced small uredia on this variety.

No explanation can be given as to the mechanism involved in this change from a type 1 to a type 4 infection; but that it was an irreversible change was shown by a selfing of the above-mentioned culture of race 8 in which the F_3 cultures studied, 52 in all, produced the type 4 infection on Sevnothree.

In the other 84 cultures comprising the F_2 population, there did not seem to be any visible difference in infection type on Sevnothree. The uredia conformed to type 1 infection except when the temperature of the greenhouse rose excessively during the early stages of pustule formation, in which case a considerable inequality in size was noted among the uredia produced on each leaf. This reaction to temperature, however, is not peculiar to the cultures under study but is characteristic of all races that produce the type 1 infection on Sevnothree.

In connection with the type 1 infection on this variety, there appears to exist between host and parasite a rather delicate balance that renders this infection type more subject to the influence of environment than the gene-controlled infection types on White Tartar and Richland.

The selfing of the two F_2 cultures, race 3 red and race 4 orange (see Fig. 2). showed that the supposed cytoplasmic effect noted in the F_1 and F_2 generations persisted equally in the F_3 generation. The infection types produced on Sevnothree in these studies were identical with those described above for the F_2 generation.

Inheritance of Infection Type on Sevnothree in Crosses Between Races 1 and 2

It was originally planned to study an F_2 generation of the cross 7 orange × 11 red as well as the reciprocal cross 11 red × 7 orange. This study would have enabled a comparison to be made between an F_2 generation derived from an F_1 hybrid producing a 4 type infection on Sevnothree and an F_2 generation derived from a reciprocal F_1 hybrid producing a 1 type infection on that variety. Only the latter study, which has been reported above, was accomplished.

With a view to making a similar comparison, crosses were made between races 1 and 2, which resemble races 11 and 7, respectively, in the infection types produced on Sevnothree, i.e., race 1 produces a type 1 and race 2 a type 4 infection. The two races produced identical infections on the other two differential hosts, White Tartar and Richland, and were, as far as was known, pathogenically identical except for the difference in infection type produced on Sevnothree. They differed, however, appreciably in urediospore color. Race 1 produced uredia of normal red color, whereas race 2 produced grayish-brown uredia. Prior to crossing, nothing was known about the breeding behavior of race 1 but race 2 had been subjected to a selfing study that had shown it to be homozygous for color of uredia and for pathogenicity.

The results of the crosses and subsequent progeny studies are shown in Fig. 3. The F_1 hybrid arising from the race 1 haploid pustule that received race 2 pycniospores (race 1 × race 2) was pathogenically identical with the race 1 parent but produced uredia of a slightly duller red color. The F_1 hybrids arising from the three race 2 haploid pustules that received race 1 pycniospores (race 2 × race 1) were pathogenically the same as the race 2 parent but produced uredia of a rather light red color instead of the grayish-brown characteristic of the race 2 parent.

Progeny studies were carried out with two of the hybrids as is shown in Fig. 3. In neither case was any visible segregation for pathogenicity observed in the F_2 generation. The selfing of race 1 gave rise to 23 F_2 cultures of which 12 arose from as many pustules of aecia and 11 from single aecia selected at random. These 23 cultures were pathogenically alike and conformed closely to race 1 but differed among themselves in the color of the uredia, 17 being classified as red and six as grayish-brown. The selfing of race 2—the hybrid

derived from pycnial pustule (1) of the cross race 2 × race 1—produced seven F_2 cultures originating from seven different pustules of aecia. These appeared to be pathogenically alike and were all identified as race 2. Six of the cultures produced red uredia but one formed grayish-brown uredia similar to those of the grayish-brown grandparental race.

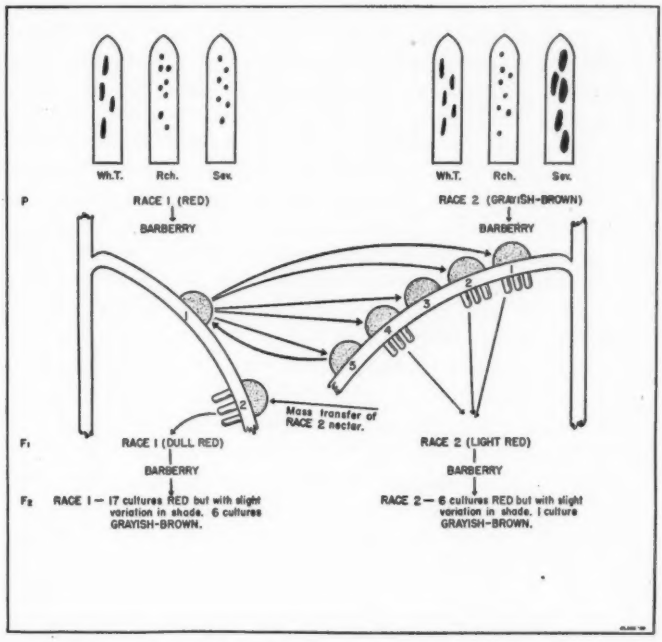

FIG. 3. *Crosses between races 1 red and 2 grayish-brown and subsequent progeny studies. Nectar was transferred from pycnial pustule (1) of race 1 red to pycnial pustules (1), (2), (3), (4), and (5) of race 2 grayish-brown and a reciprocal transfer was made from pustule (5) of race 2 grayish-brown to pustule (1) of race 1 red. Pustule (2) of race 1 red received a mass transfer of nectar from several pustules of race 2.*

Discussion

It is established in the present paper that pathogenic characteristics in oat stem rust are inherited in much the same manner as in wheat stem rust (4). The ability of the rust to develop certain types of infection on given host varieties is conditioned by genes behaving in accordance with Mendelian laws. In the cross between races 7 and 11, the type 2 infection expressed on the variety White Tartar is a dominant character, the type 4 infection being the corresponding recessive character. Similarly, on the variety Richland, the type 1 infection is dominant to the type 4. In both cases, the inheritance in F_2 was apparently governed by two pairs of factors complementary to each other.

As in wheat stem rust, there is evidence that not all pathogenic characters are under the governance of Mendelian, i.e., nuclear, factors. In wheat stem rust, it has been shown (5, 6, 7) that the inheritance of certain infection types expressed on the variety Marquis could be explained only on the assumption that the cytoplasm of the "maternal" parent race influenced the type of infection produced by the F_1 hybrid. The selfing of the F_1 hybrid showed, furthermore, that this influence persisted to a large degree in the F_2 generation, which showed a decided, though not absolute, uniformity in the infection type on Marquis while at the same time there was clear evidence for segregation in the pathogenic characters expressed on certain other wheats. In the case of crosses between races of oat stem rust, it has been shown previously (3) that a similar influence of the maternal cytoplasm must be postulated to account for the inheritance in F_1 of the infection types of races 7 and 11 on the variety Joanette Strain and its selection Sevnothree. In the present paper, evidence is presented to show that hybrids between races 1 and 2, which correspond respectively to races 11 and 7 in types of infection on Joanette Strain and Sevnothree, show in F_1 the same behavior on these oat varieties as did hybrids between races 11 and 7.

It is now for the first time demonstrated that in oat stem rust the cytoplasmic influence affecting the infection types on these two varieties persists in the F_2 generation in much the same manner as did the cytoplasmic influence expresssd by wheat stem rust races on Marquis.

No new evidence is presented to explain the mechanism of this type of inheritance. It must be supposed either that there are factors of some sort that are transmissible through the cytoplasm of the maternal parent rust or that there are genic (nuclear) factors that operate differently in one cytoplasm than in another. In the latter case, the factors would not be cytoplasmic but rather nuclear factors influenced by the cytoplasm. Whatever the nature of these factors they are evidently not immutable. The occurrence, in one of the 85 F_2 cultures of the cross race 11 $\times$ race 7, of a clone with type 4 instead of type 1 infection on Sevnothree suggests a mutation in the factors governing the infection type on this host. That this mutation was no transitory change was shown by a selfing of this clone (culture No. 5 in Fig. 2) whose progeny displayed the new characteristic in all the 52 F_3 cultures studied.

The demonstration of the cytoplasmic inheritance of the type 1 and type 4 infections on Joanette Strain and Sevnothree in the crosses discussed in the present paper raises the question of whether the type 1 and type 4 infections on these varieties are in all instances similarly inherited. It is possible to arrive at some opinion in this connection by considering the various selfing studies carried out with physiologic races exhibiting these infection types. If, in a given physiologic race, the cytoplasm conditions pathogenicity and, if there is no appreciable effect through the agency of nuclear factors, it should follow that there would be no visible variation in infection type in the progeny. The chief difficulty in the way of this line of reasoning is that, in any strain of

rust for which cytoplasmic inheritance has not been demonstrated by reciprocal crosses, it is possible that uniformity in the progeny may be due to homozygosity of nuclear genes rather than to cytoplasmic control. But all races would not be expected to be homozygous for nuclear genes, and, if a number of races are studied and all are found to be homozygous for the characters in question, there would be some reason to suppose that cytoplasmic agencies rather than nuclear factors were at work.

To test this idea the available records of selfing studies with physiologic races of oat stem rust were examined with particular attention to the infection types produced on Joanette Strain. The results of this survey are summarized in Table V.

Of the 11 different clones of races 2, 6, 7, and 8, which produced type 4 infection on Joanette Strain, it will be seen that nine produced a uniform progeny in this respect, whereas two showed a tendency to produce type x infection in some or all of their progeny. Only one clone, race 6 orange No. 2, showed any real indication of segregation. In the case of the other clone that showed indications of type x infection in its progeny, race 8 No. 3, there was no apparent segregation but all isolates appeared to be on the border line between types 4 and x. It seems justifiable to conclude that, as far as the type 4 infection is concerned, there is no definite segregation of infection types.

With respect to the type 1 infection, the available information is more scanty and the evidence less clear. Only three clones were studied, one of race 3 and two of race 11. One of these, race 11 No. 1, showed no evidence of segregation. The second clone of race 11 gave rise to a progeny of 52 isolates identical in all respects except that 40 of them produced type 1 infection on Joanette Strain whereas 12 produced type x infection. This conforms closely to the 3 : 1 ratio, which might be expected from the governance of a single pair of Mendelian factors. The third clone studied, race 3, produced two isolates with type x infection out of a total of 25 isolates. In view of these results, it seems likely that inheritance of the type 1 infection is not wholly conditioned by non-nuclear agencies.

The inheritance of the third type of infection that occurs on Joanette Strain, type x, has not been studied to any great extent. Results of crosses of type x races with type 1 and with type 4 races have indicated a maternal inheritance of the type x infection (3). In Table V are recorded selfing studies with four clones of races that produced type x infection on Joanette Strain. Three of these, race 5 No. 2, race 10, and race 12, produced the type x infection in all their progeny—a fact that seems to show conclusively that the type x infection in these clones is not the result of heterozygosity for nuclear factors. The fourth clone, race 5 No. 1, produced type x infections in 95 out of 106 isolates and type 4 infections in the remaining 11 isolates. A possible interpretation is that nuclear as well as cytoplasmic factors affected pathogenicity in this clone.

As far as present available evidence permits a conclusion to be drawn concerning the inheritance of the infection types on Joanette Strain, it would seem

TABLE V

INHERITANCE OF INFECTION TYPE ON JOANETTE STRAIN IN THE SELFING OF OAT STEM RUST RACES NOT DERIVED FROM CROSSES

Race selfed	Infection on Joanette Strain	Races in progeny*	Infection types on differential hosts		
			White Tartar	Richland	Joanette Strain
2 No. 1	Type 4	2 (23)	2	1	4
2 No. 2	Type 4	2 (3)	2	1	4
2 orange	Type 4	2 (15)	2	1	4
2 gray	Type 4	2 (13)	2	1	4
6	Type 4	6 (14)	4	4	4
		7 (3)	4	1	4
		8 (12)	2	4	4
6 orange No. 1	Type 4	6 (38)	4	4	4
6 orange No. 2	Type 4	6 (33)	4	4	4
		13?(3)	4	4	4 to x
7 orange	Type 4	7 (43)	4	1	4
8 No. 1	Type 4	6 (7)	4	4	4
		7 (1)	4	1	4
		8 (15)	2	4	4
8 No. 2	Type 4	6 (2)	4	4	4
		8 (4)	2	4	4
8 No. 3	Type 4?	6?(6)	4	4	4 to x
		8?(8)	2	4	4 to x
3	Type 1	3 (15)	4	1	1
		4 (8)	4	4	1
		12 (1)	4	1	x
		13 (1)	4	4	x
11 No. 1	Type 1	11 (25)	2	4	1
11 No. 2	Type 1	10 (12)	2	4	x
		11 (40)	2	4	1
5 No. 1	Type x	2 (11)	2	1	4
		5 (91)	2	1	x
		10 (2)	2	4	x
		12 (2)	4	1	x
5 No. 2	Type x	5 (29)	2	1	x
10	Type x	10 (23)	2	4	x
12	Type x	12 (14)	4	1	x

* *Number of isolates in parentheses.*

that the results of crosses and selfing studies with hybrids demonstrate that the type 4 and type 1 infection may be governed by cytoplasmic factors. The results of selfing studies indicate that in some clones, at least, nuclear factors may affect inheritance, particularly of the type 1 infection. The rather limited evidence available from crossing studies suggests that the inheritance of the type x infection may also be cytoplasmically conditioned but here, again, the selfing studies appear definitely to preclude the view that nuclear factors never enter into the inheritance of this infection type.

Acknowledgments

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NATURAL PINE HYBRIDS IN ALBERTA[1]

By E. H. Moss[2]

Abstract

In central and northwestern Alberta, where the ranges of *Pinus contorta* var. *latifolia* and *P. Banksiana* overlap, there is abundant field evidence of hybridism between these species. Numerous trees have been found with cone and other characters more or less intermediate between those of the two species. These trees are generally found in proximity with, but rarely apart from, the putative parent species. Various diagnostic characters for the species are briefly evaluated in interpreting the polymorphic hybrid population. Characters of the mature seed cones are used to distinguish several pine types in the region and as a basis for a discussion of problems requiring further investigation.

Introduction

This paper deals with the occurrence in Alberta of the two closely related species, *Pinus contorta* Loudon var. *latifolia* Engelm. (*P. Murrayana* Balf.) and *Pinus Banksiana* Lamb. (*P. divaricata* Du M. de C.), and also with field evidence of extensive hybridization between these species where their ranges overlap.

Pinus contorta latifolia, commonly known as lodgepole pine, has an eastward extension in Alberta as shown on the accompanying map (Fig. 1). The lines representing the occurrence of this species, both the general eastward range and the outliers associated with certain hills, follow closely those published by Halliday and Brown (6), except for the region between Edmonton and the Peace River area. In this region, the line has been drawn to conform to the results of the present study. *Pinus Banksiana*, commonly called Banksian or jack pine, extends westward and southward in Alberta as shown by the broken line (Fig. 1), this line being approximately that published by Halliday and Brown (6), except where altered in the Edmonton – Peace River region in accordance with data of the present investigation.

In the overlapping region, where both species grow, there are also found many trees with characters intermediate between those of the two species. The only published report of this situation appears to be in the form of a personal communication by the present writer to Dr. H. M. Raup (12) in which the writer stated his observations of 1941 as follows: "*Pinus Banksiana* seems to prevail at Smith and on the north shore of Lesser Slave Lake opposite Widewater and Canyon Creek. Between Lesser Slave Lake and Peace River, *P. Banksiana* and *P. Murrayana* were observed. At Peace River town both species grow intermixed, with jack pine predominating. Trees showing intermediate characters were rather common in the Slave Lake – Peace River region". During the past four years the writer has made brief studies on the pines in numerous areas throughout a considerable portion of the region. These studies, presented in this paper, are to be regarded as only a preliminary

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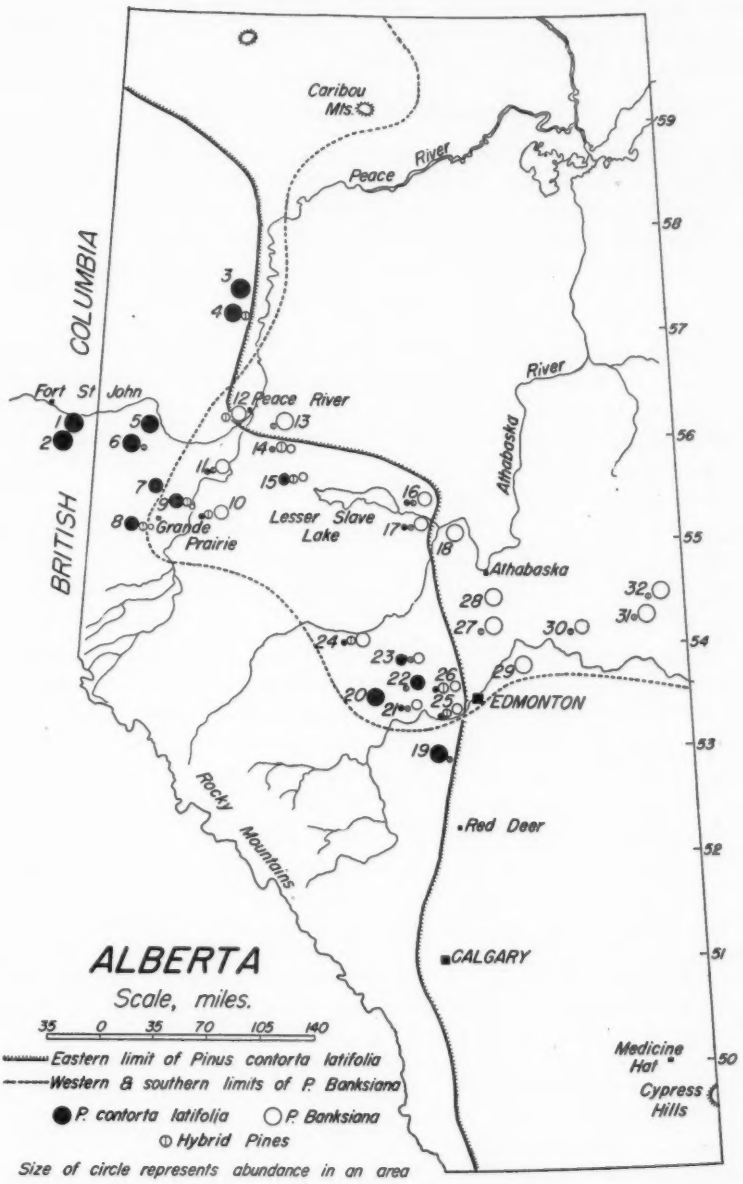


FIG. 1. *Outline map of Alberta, showing the transition from lodgepole to jack pine.*

account of the leading pine types and their occurrence in the region. They serve also to suggest various ecological, phytogeographical, and genetical problems worthy of critical investigation.

Contrasting Features of the Species

The following discussion is based on: (*a*) descriptions in standard manuals, including those of Britton (3), Dallimore and Jackson (4), Elwes and Henry (5), Hough (8), Morton and Lewis (10), Rehder (13), Rydberg (14), Sargent (15), Sudworth (18); (*b*) critical evaluation of characters in *Pinus* by Shaw (16); (*c*) recent studies by the writer.

Tree Habit

Lodgepole pine in Alberta generally grows in dense stands where it develops a tall, straight, clean trunk. Open stands of the pine are not uncommon in the region studied. The trees of these stands have broad crowns and usually retain their branches near the trunk base. The ends of the branches show a pronounced tendency to curve upward, contrasting with the generally drooping branches of wide-spreading jack pine.

Jack pine, in most of the areas studied, exhibits the wide crown and rather scrubby growth commonly regarded as characteristic of this species. But, as has been reported for various other parts of Canada, so in Alberta, dense stands of this pine have trees with tall, slender, clean trunks and small crowns.

Therefore, while general tree habit cannot be relied upon as a diagnostic feature, the semi-erect branch form of the lodgepole contrasts with the drooping branches of the jack pine, at least in older trees with wide crowns. This difference in branching habit has been observed in lodgepole and jack pine growing side by side, thus indicating that it is an inherent rather than a habitat response. Pines with various of the intermediate characters described in this paper usually show also an intermediate branch habit.

Bark of the Trunk

According to most authorities, the outer bark of the lodgepole pine is darker in color, and characterized by finer and more uniform scales, than that of the jack pine. Whereas the lodgepole bark forms small, loosely appressed scales, the jack pine splits into irregular, scaly ridges and furrows. While these differences appear to be fairly constant for the two species in our region, definite conclusions as to bark characters of the intermediate pine types are not indicated but might be reached through an extensive investigation.

Foliage

Lodgepole pine is described as having considerably longer, less twisted and less divergent needles than jack pine, and arranged in more dense clusters toward the ends of the twigs. In our experience, these are fairly good

diagnostic features for the species. While we have made no critical studies on needle length, intermediates between the species appear generally to have the jack pine type of foliage.

Leaf Anatomy

Harlow (7) employs shape of leaf epidermal cells to distinguish these two species. The epidermal cells of lodgepole pine are said to appear nearly square, those of jack pine rectangular in transverse section. In our experience this is wholly unreliable as a distinguishing feature. Examination of numerous collections has failed to reveal any constant difference in the epidermal cells of the two species. This anatomical character seems therefore to have no value for the diagnosis of intermediate forms.

Staminate Cones and Pollen Characters

The pollen sacs of lodgepole pine are said to be orange or orange-red, those of jack pine yellow in color. Since most of our field studies were done after the staminate cones had matured, we are unable to express an opinion as to the reliability of this feature; nor have we been able to use this as a criterion for determining affinities of intermediate pine types.

We have evidence that where the two pine species grow together, jack pine sheds its pollen a few days in advance of lodgepole. For instance, at Heatherdown (Area No. 26), on June 2, 1948, typical jack pine was shedding pollen, while the pollen cones of lodgepole were still greenish. A tree of an intermediate type (similar to that shown in Fig. 9) was just beginning to shed pollen, thus indicating a closer affinity with jack pine than with lodgepole. Pollen of this intermediate pine type was found to be about 50% normal in appearance and 50% distorted and shrunken, while pollen of typical jack and lodgepole trees, collected at the same time, showed practically no distortion of grains.

Seed Cones

Position of Cones on Stem

Certain authorities, including Sargent (15), Elwes and Henry (5), place *Pinus contorta* and *P. Banksiana* in different Sections of the genus, on the understanding that *P. contorta* bears its cones subterminally on uninodal shoots, while *P. Banksiana* has its cones lateral in position on multinodal shoots. Shaw (16) established that these characters should not be employed for grouping species of *Pinus*, "for Pines are not sharply divided into multinodal and uninodal species, and no exact segregation of them based on this difference is possible". Actually Shaw describes both *P. contorta* and *P. Banksiana* as multinodal, although he emphasizes that "the multinodal shoot is never invariable in a species, but is rare, common or prevalent". Unfortunately, cone position was used later by Sterrett (17) to distinguish these two species. Still more unfortunate is Sterrett's statement that jack pine has the cones subterminal and lodgepole pine has the cones lateral, which reverses the distinction emphasized by the earlier taxonomic treatments. Sterrett's statement led us to make a few counts on local plantations of typical lodgepole

and jack pine. The result for lodgepole was seven lateral to eight subterminal; for jack pine, 36 lateral to one subterminal. These results are in accord with Shaw's conclusions. It is probable, therefore, that data on cone position have little or no value for an interpretation of our intermediate forms of pine.

Cone Characters

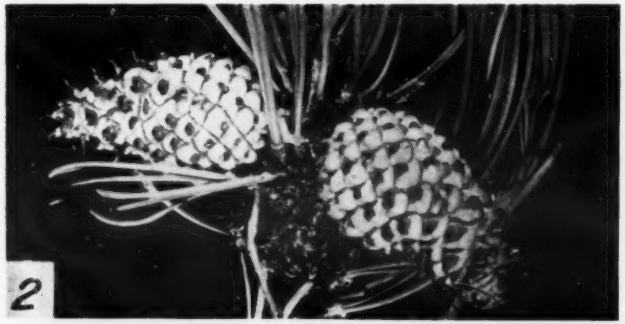
Features of the mature seed cones are now generally considered to be of prime importance in distinguishing lodgepole and jack pines. Most authorities emphasize these differences somewhat as follows. The mature cone of lodgepole pine is spreading or reflexed, ovoid or conical in shape, the scales conspicuously umbonate, each scale armed with a minute recurved prickle (Fig. 2). The mature cone of jack pine is erect (directed toward the apex of the shoot), strongly incurved or slightly spreading, the scales variously thickened (the outer and lower, large, mammiform) and unarmed (Fig. 3). This characterization is supported by the present study.

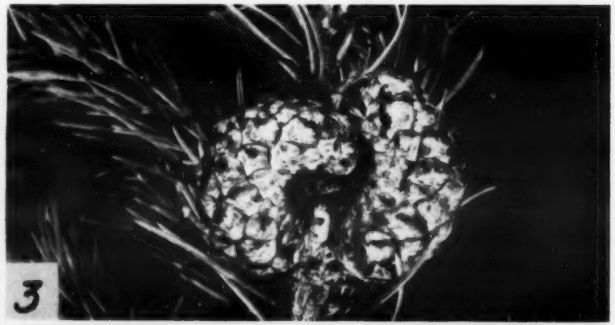
Actually, a number of the manuals do not draw the foregoing sharp distinctions between the two species, and probably for the following reasons. (*a*) Numerous intermediate forms do occur, such as those of the overlapping region dealt with in this paper. (*b*) Quite outside the overlapping region, there are known to be forms of these pines that do not conform to either of the descriptions given above. The most baffling type of pine encountered in this investigation has spreading (horizontal), straight, smooth cones (Fig. 4). This form of pine is known to occur far eastward in Canada. Mr. A. J. Breitung (letter of Aug. 17, 1947) informs me that he has cones of this type from Nipawin, Sask., and Mr. J. S. Rowe (letter of Jan. 22, 1948) has supplied information, including photographs, of the same type growing in Riding Mountain Park, Man. For our present purpose this pine type is regarded as a form of *Pinus Banksiana*, as also is the type shown in Fig. 5, which is intermediate in cone characters between the former and typical jack pine.

Our somewhat arbitrary decision to regard this type (Fig. 4) as a form of jack pine, rather than an intermediate form, is based mainly on the following considerations: (*a*) its occurrence far eastward beyond the present range of lodgepole pine; (*b*) considerable evidence that it is more prevalent in stands composed chiefly of jack pine than in mixed stands of jack and lodgepole pines; (*c*) its branching habit and needle characters are, in our experience, those of typical jack pine. Admittedly, there are good reasons for adopting the alternative view, viz. that this type of pine is a lodgepole–jack hybrid. This aspect of the subject is discussed more fully later in the paper.

In spite of certain complications, such as those just indicated, cone characters are judged to be the best readily available diagnostic feature for these pines. The following presentation of intermediates (hybrids) between the two species is based, in large part, on characters of the mature seed cones.

It should be noted that the mature cones show remarkably little variation in any particular tree, regardless of the type.





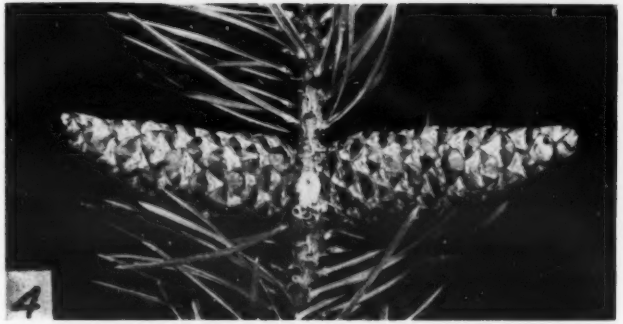
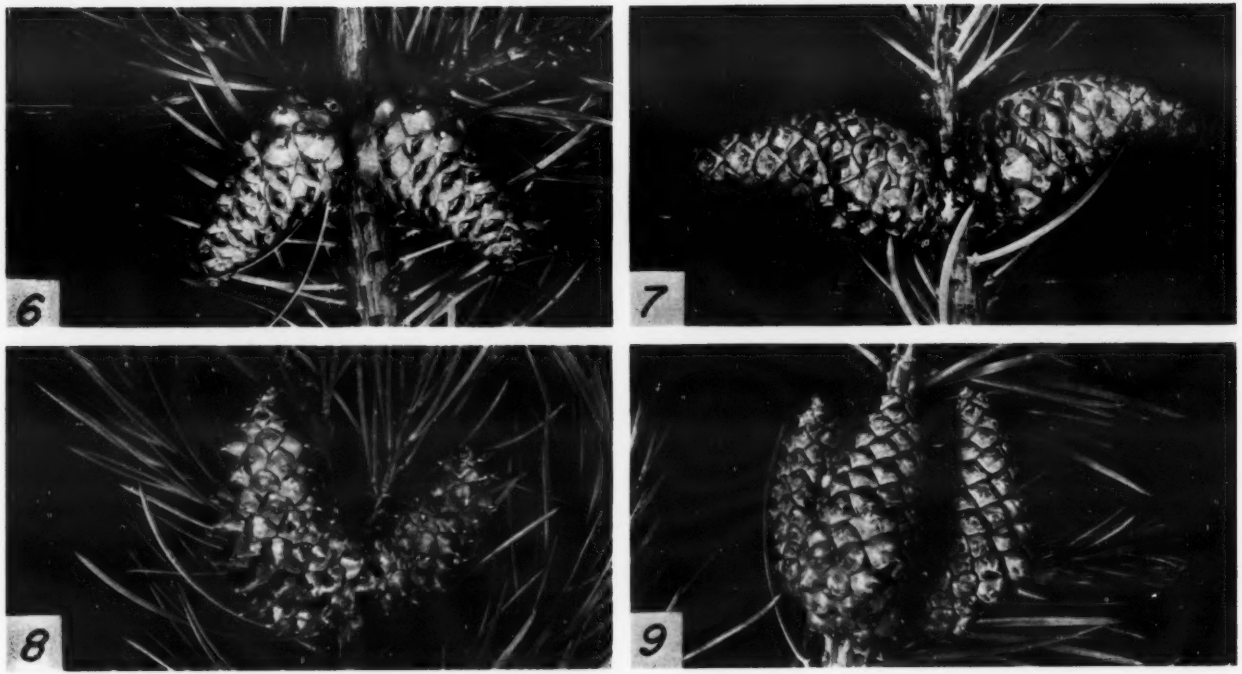


FIG. 2. *Pinus contorta latifolia; typical cones; approx.* × *0.8.*
FIG. 4. *P. Banksiana; straight, horizontal cones; approx.* × *0.8.*

FIG. 3. *Pinus Banksiana; typical cones; approx.* × *0.8.*
FIG. 5. *P. Banksiana; semi-erect cones; approx.* × *0.8.*



FIGS. 6 to 9. *P. contorta latifolia* × *P. Banksiana; Fig. 6, reflexed, smooth; Fig. 7, horizontal, smooth, with recurved tip; Fig. 8, erect, incurved, spiny; Fig. 9, erect form, weak but persistent spines. (All figures are approximately* × *0.8.)*

Hybrids in the Transition Region

Where the ranges of lodgepole and jack pine overlap, the two species and the many intermediate forms occur in varying proportions from place to place. Of a large number of stands examined, 32 are described below and shown on the outline map (Fig. 1). The circles on the map give only an approximate idea of the relative abundance of the species and hybrids. The largest circles represent a very high frequency; the smallest circles indicate a rare occurrence. The considerable space occupied by the circles on the map is not to be taken as a measure of pine forest in the region. Actually, pine stands are relatively uncommon in most parts of the region covered by this investigation and comprise only a small fraction of the total forest cover.

The main types of intermediates (hybrids) are as follows:

(*a*) Illustrated in Fig. 6; cone reflexed and smooth; like typical lodgepole in shape, resembling jack pine in lacking prickles; generally associated with the jack pine type of foliage; a rather rare type.

(*b*) Shown in Fig. 7; cone nearly horizontal, with a recurved tip, smooth or with weak prickles; resembles the form of jack pine (Fig. 4) with straight, horizontal cones and may conceivably be more closely related to that form than to typical lodgepole; a fairly common type.

(*c*) Shown in Fig. 8; cone erect, markedly incurved and with strong prickles; like lodgepole in being prickly but close to typical jack pine in shape; sometimes on trees with an upright branch habit and long needles crowded near the branch tips, evidence of lodgepole ancestry; a rather common type.

(*d*) Illustrated in Fig. 9; cone erect, somewhat incurved, and with weak prickles; usually associated with the jack pine branching habit and type of foliage; common in most parts of the transition region.

(*e*) Not illustrated; cone semi-erect and prickly; found occasionally.

(*f*) Not illustrated; cone semi-erect, tip recurved; probably close to jack pine; fairly common.

(*g*) Not illustrated; cone reflexed, weak spiny; evidently close to lodgepole; apparently rare.

(*h*) Not illustrated; exceptionally large cone, semi-erect, smooth; on trees with branching and foliage approaching the lodgepole type. Mention may be made here that trees with enormous, erect, incurved, smooth cones, and vegetative characters of the jack pine type have been seen; these appear to be typical jack pine in every way, except for the unusually large cones.

It is evident that almost every conceivable combination of lodgepole and jack pine cone characters has been found in the region. Trees with these intermediate characters are most abundant, (*a*) in the central parts of the overlapping region, (*b*) in stands where both lodgepole and jack pine occur. Conversely, trees with intermediate characters are relatively rare, or absent, (*a*) near the range limit of each species, and beyond that limit, (*b*) in stands

where only one of the species has been found. These observations are believed to lend strong support to the view that those pines with intermediate characters are indeed hybrids with lodgepole and jack pine ancestry.

Data for Pine Stands

No. 1. Dawson Creek – Fort St. John, B.C. An area with considerable variation in soil and topography. Lodgepole only seen; trees mostly tall and straight, a few with somewhat spreading habit.

No. 2. Progress, B.C., about 25 mi. west of Dawson Creek. Typical lodgepole pine, growing with poplars and white spruce.

No. 3. Hawk Hills, Alta. Lodgepole only observed; intermixed with poplars and spruce.

No. 4. North of Meikle River. Chiefly lodgepole. A few intermediates (Figs. 8, 9).

No. 5. Blueberry Mountain. Variable soil and topography. Lodgepole only seen.

No. 6. About 25 mi. west of Spirit River. Nearly all typical lodgepole. Very few intermediates (Fig. 9).

No. 7. Saddle (Burnt) Hills. Lodgepole only seen.

No. 8. Grande Prairie. About four miles southwest of Grande Prairie, near the Wapiti River; a sand-hill area. Lodgepole abundant, most with reflexed, some with horizontal, cones. Intermediates (Figs. 8, 9) scattered throughout. Jack pine occasional and localized.

No. 9. Kleskun Hill, about 12 mi. northeast of Grande Prairie. Hilly, "badland" area; small groves and isolated trees. Lodgepole pine abundant. Intermediates (Fig. 9) occasional. Jack pine very rare; one tree seen.

No. 10. Smoky River, east of Grande Prairie. Rough topography. Pines on slopes and flat areas in valley. Lodgepole occasional. Intermediates, various types (chiefly Fig. 9), scattered. Jack pine, typical form, abundant. Jack pine (Fig. 4) rare.

No. 11. Southeast of Codesa. Level, sandy area. Typical lodgepole, rare. Intermediates rare. Jack pine, typical form, abundant. Jack pine (Fig. 4) scattered.

No. 12. Peace River (town). Sandy and gravelly flats of river valley. Trees of various growth forms, mainly with wide crowns. Intermediates, various forms, including those illustrated (Figs. 6 to 9), comprise somewhat over one-quarter of the stand. Typical jack pine, about one-half of the trees. Other forms of jack pine, about one-fifth of the stand.

No. 13. Harmon Valley, 20 mi. east of Peace River. Sandy area, rolling topography. Intermediates (Figs. 8, 9) occasional. Jack pine, typical, abundant; (Fig. 4) occasional.

No. 14. McLennan–Nampa. Mostly heavy soil and flat topography. Lodgepole and jack pine frequent. Intermediates, including types illustrated (Figs. 7, 8, 9), more abundant than either of the species. Growing with aspen, balsam poplar, and white spruce.

No. 15. Triangle. Mainly a low, flat area. Lodgepole, intermediates of various kinds, and jack pine are all well represented. Pines tall and slender, forming pure stands locally, otherwise with poplars and white spruce.

No. 16. Lesser Slave Lake, at east end. Sand dune area. Lodgepole rare. Intermediates rare. Jack pine, typical, abundant; (Fig. 4) occasional.

No. 17. Eight miles southeast of Slave Lake (town). Low sandy area, bordering peat bog. Lodgepole, typical, occasional to scattered. Intermediates occasional. Jack pine, typical, scattered; (Fig. 4) occasional.

No. 18. Smith. Sand hills. Jack pine, typical, abundant; (Fig. 4) occasional.

No. 19. Battle Lake, west of Pigeon Lake. Fairly low area with variable soil, some heavy. Lodgepole, typical, abundant. Intermediates, similar to Fig. 9, but less incurved and with stronger spines, rare.

No. 20. Lobstick. Low flat area. Dense stand of pine and black spruce. Typical lodgepole, abundant.

No. 21. Wabamun Lake, near Seba. A fairly flat, sandy area. Pines mainly in open stands. Lodgepole occasional. Intermediates occasional. Jack pine, typical, common; (Fig. 4) rare; (Fig. 5) occasional.

No. 22. Evansburg. Sandy to heavy soil. Lodgepole abundant. Intermediates (Fig. 8), rare.

No. 23. Sangudo. Somewhat sandy area. Lodgepole frequent. Intermediates occasional. Jack pine frequent.

No. 24. Whitecourt. Sandy region; flat to rolling topography. Lodgepole occasional. Intermediates scattered, including those shown in Figs. 6 to 9 and other forms, e.g. reflexed, weak spiny; semi-erect and spiny. Jack pine, typical, frequent; (Fig. 4) rare.

No. 25. Graminia, about 20 mi. southwest of Edmonton. Sand-hill area. Lodgepole rare. Intermediates of many kinds, including types shown in Figs. 6 to 9, scattered. Jack pine, typical, frequent; (Fig. 4) rare.

No. 26. Heatherdown, about 25 mi. northwest of Edmonton. Gravelly, hilly area. Lodgepole rare. Intermediates (Figs. 7 to 9) and other kinds, scattered. Jack pine, typical, scattered; (Fig. 4) occasional; (Fig. 5) rare.

No. 27. Nestow. Sand-hill area. Intermediates (Figs. 6, 7) rare. Jack pine, typical, abundant; (Fig. 4) occasional; (Fig. 5) rare.

No. 28. Rochester. Sand-hills. Jack pine, typical, frequent; (Fig. 4) scattered; (Fig. 5) occasional; also a few trees with exceptionally large cones, otherwise typical jack pine.

No. 29. Bruderheim. Sand-hill area. Jack pine, typical, abundant; (Fig. 4) occasional (1 to 2% of the latter type).

No. 30. Bellis. Somewhat sandy, flat to gently rolling, area. Intermediates occasional; various types, including those shown in Figs. 6, 7. Jack pine, typical, frequent; (Fig. 4) occasional; (Fig. 5) occasional to scattered.

No. 31. St. Paul–Bonnyville. Sandy, flat area. Intermediates (Fig. 7) rare. Jack pine, typical, abundant; (Fig. 4) occasional; (Fig. 5) occasional.

No. 32. Beaver River. Sandy, rolling area; near river valley. Intermediates (Fig. 7) rare. Jack pine, typical, abundant.

Discussion

Range Limits of the Species

On the map (Fig. 1) the heavy lines are believed to represent fairly accurately the range limits of lodgepole and jack pine as understood by Halliday and Brown (6) and modified locally by the present writer. Further observations will doubtless necessitate certain changes in these lines, especially in the relatively unknown areas between Whitecourt and Grande Prairie, northeast of Lesser Slave Lake, and throughout the more northern region.

Ecological Considerations

Our field data point to the conclusion that lodgepole is more prevalent than jack pine on the heavier soils, while jack pine is much more abundant on sandy areas. Striking exceptions to the rule do occur, for instance, the extensive growth of lodgepole pine on sand hills southwest of Grande Prairie. It is probably significant that this area is virtually in the lodgepole pine belt and close to the western limit of jack pine. Worthy of note too is the fact that lodgepole and jack pine are occasionally found growing side by side even on sandy areas. Both species appear to thrive on gravelly or stony knolls and river flats. Conclusions concerning the relation of the intermediate forms to site are not clearly indicated. It is obvious that an adequate understanding of adaptational aspects of these pine types must await critical investigation.

Lodgepole pine is commonly associated with other tree species, principally *Picea glauca* (Moench) Voss, *Populus tremuloides* Michx., and *P. Tacamahacca* Miller, these occurring in varying proportions and often as dense stands of tall, straight trees. Jack pine usually forms open stands, with relatively few trees of the associated species, *Populus tremuloides* and *Betula papyrifera* Marshall, these being small and scrubby. Where jack pine occurs on the heavier soils, it is generally associated with white spruce, poplars, lodgepole pine, and hybrid pines, all with a slender habit and in close stands.

Phytogeographical Aspects

It is probable that lodgepole and jack pine have migrated into the overlapping zone, from west and east respectively, during a rather recent period. On the other hand, the view that both species have been long established in the region finds some support in their distribution, which is at once extensive and discontinuous. It is possible too that at an earlier period the pines had an even greater coverage there than at the present time. Of interest in this connection is Raup's interpretation (11) of certain pine–spruce relationships in the country between Athabaska and Great Slave Lakes. Raup suggests that the earliest postglacial forests were of white spruce arranged in an open, parklike association and that, with the warming of the climate and decrease of ground frost, the less tolerant pine could enter and compete successfully in the spruce stands on sandy areas. With further amelioration of climate and improvement in the moisture and humus conditions, there was a tendency for spruce to suppress the pine and again to dominate these areas. Changes along these lines may have occurred in the sand-hill parts of our region, though nearly all of these appear to be held at the pine stage, probably as a result of frequent burning. Similar changes may have taken place on flatter areas and on heavier soils, leading to the mixed spruce–pine–poplar forest of the present time. Involved here are *Populus tremuloides* and *P. Tacamahacca*. Like the pines, the poplars tend to be succeeded by white spruce, but are favored by occasional burning. There is a distinct possibility, therefore, that the pine species at one time dominated larger areas in the region.

Of interest here is Hultén's view (9) that the "Linnaean species" of the boreal flora originated in the last great interglacial or earlier and that the present areas of boreal biota are, on the whole, reductions from the areas that had developed already during interglacial times. Accordingly, lodgepole and jack pine may be assumed to have occurred as distinct species and with overlapping ranges during interglacial times. If so, they must have formed hybrids even as they do now. Moreover, during a long interglacial, certain of the transition forms were probably segregated in response to special conditions or by geographical barriers or otherwise. The possibility of such "hybrid" forms surviving glaciation in refugia and later returning to the region is of interest in connection with the aberrant jack pine types (Figs. 4, 5). While this suggestion may be highly speculative, it should not be dismissed until exploration has established the absence of aberrant pine types from those areas southeastward from Manitoba that were presumably supplied with pines from the Wisconsin refugium.

Aspects of Hybridism

The criteria for hybridism employed in this study are essentially those approved by Allan (1) for wild species hybrids. These criteria may be stated briefly as follows: the occurrence of an assemblage of forms more or less intermediate between the putative parent species, these forms comprising a

graduated series linking one species with the other and generally found in proximity with, but rarely apart from, the parent species.

The occurrence of pine hybrids well beyond the present eastern limit of lodgepole pine requires an explanation. This is at hand if the hypothesis of a more easterly range for this species in earlier postglacial time is accepted. An alternative hypothesis is in terms of the eastward migration of certain hybrids well adapted for the prevailing conditions. Of interest in this connection is the phenomenon of introgressive hybridization that has been demonstrated for several groups of plants through the work of Anderson (2) and others. It may well be that we have introgression of lodgepole into jack pine, but this has yet to be established by biometric analysis.

As already suggested the aberrant jack pine types (Figs. 4, 5) may actually be hybrids derived from a postglacial union of lodgepole and typical jack pine. The occurrence of at least one of these types (Fig. 4) as far east as Manitoba seems to make considerable demands upon a migration hypothesis, especially if this assumes lodgepole pine to have extended no farther east than its present range. An investigation of the pines throughout the region using the techniques employed in introgression studies would doubtless clarify our understanding of the whole situation.

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YIELD AND PROTEIN CONTENT OF WHEAT AND BARLEY

I. INTERRELATION OF YIELD AND PROTEIN CONTENT OF RANDOM SELECTIONS FROM SINGLE CROSSES[1]

By M. N. Grant[2] and A. G. McCalla[3]

Abstract

Yield and protein content of 100 random selections from single crosses of wheat and barley were determined for three years. Results of each test showed highly significant differences in yield and protein content of selections. There was a highly significant negative correlation between yield and protein content in each test, the values for r_{py} varying from $-.483$ to $-.806$. In general, the relationship was improved when mean values from several tests were correlated. The behavior of individual selections under a wide range of environmental conditions was relatively constant even though wide differences occurred in both yield and protein content of individual selections.

Introduction

Data from many sources suggest that there is a significant negative association between yield and protein content of wheat and barley (1, 3), and it seems altogether likely that part of this association is, in some manner, genetically controlled. The association has great practical importance if it is consistent and close. The plant breeder will find it difficult to develop bread wheats that give maximum yields and yet have satisfactorily high protein content. On the other hand, varieties of malting barley and wheat for pastry flour will likely have high yield associated with reasonably low protein content, a condition that is desired.

Some of the early literature pertinent to this problem was reviewed by Neatby and McCalla (3) and extensive new data were presented. Since then, the most important contribution has been made by Berg (1) working with winter and spring wheats in Sweden. He used selections from hybrid populations, but these differed radically from those used in the present study inasmuch as rigid selection had been practised in earlier generations to eliminate strains that were not winter hardy, were weak, low in yield, or poor in quality. Selection for yield and quality certainly had the effect of reducing the range of yield and protein content in the selections studied. Nevertheless, Berg obtained results agreeing well with those published earlier. Correlation coefficients of $-.578$ and $-.607$ were obtained for winter and spring wheats. While he found that it is possible to combine higher yield with higher protein content, the extent to which such combination can be secured seems limited.

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While the study by Neatby and McCalla (3) was under way, it appeared that a true measure of the correlation between yield and protein content, as it pertained to breeding, could be obtained only by working with random selections from hybrid populations of wheat and barley. This paper reports the results obtained over a period of three years' study with such material.

Material and Methods

In selecting parental material for the production program, chief attention was directed toward the yielding ability and protein content of each potential parent. Obviously, greater ranges could be expected by crossing varieties that most clearly exhibited extremes in the characteristics to be studied. Although other factors were not disregarded, one of the parents used was high in yield but low in protein, while the other was low in yield but high in protein.

Barley

The barley parents selected were Trebi and Peatland. Trebi is consistently a very high yielder, but is one of the varieties lowest in protein. Peatland is a low yielder, but has usually shown very high protein content under Alberta conditions. The cross was made in 1936. The seed from the cross was grown in bulk plots until 1939 when 100 random selections were made of plants carrying F_4 seed. The selected material was planted in head rows in 1940, and in 1941 the increased seed was used in a quadruplicated yield test at Edmonton. Moisture conditions were very poor and the stand was uneven, with late selections suffering much more than early ones. Useful results were scarcely expected from this test.

Owing to labor shortages arising from the war further work had to be postponed until 1944. In 1944 and again in 1945, 100 random selections were grown at Edmonton, while in 1946, 50 of these 100 samples were grown on black loam soil at Edmonton and also at Fallis, located 50 mi. west of Edmonton, on a relatively infertile podsolic loam soil.

Wheat

The parents used in the wheat study were Bunyip and Dicklow. Both are soft white wheats: Bunyip is characterized by low yield and high protein, while Dicklow has consistently been a very high yielder but has had the lowest protein content of any variety grown under local conditions. The cross was made in 1936 and the seed grown in bulk plots until 1940, when a random selection of heads from F_4 plants was made. These were increased in 1941. One hundred of these selections were used in yield trials at Edmonton and Fallis in 1944 and 1945, while 50 of the 100 were used at each place in 1946.

Design and Production

Each field test in 1944 and 1945 was in the form of a 10 × 10 simple lattice (2) with four replicates. Standard rod-row plots with three rows and 9 in. spacing were used. Seeding of barley was at the rate of 2 bu. per acre, and of wheat at $1\frac{1}{4}$ bu. No fertilizer was used. Only the center row of each plot

was harvested. Each replicate was threshed and weighed separately but, except in one year, protein determinations were made on composite samples.

In 1946 the 50 selections were handled in the same manner except that the design used was a simple randomized block.

Weather Conditions

1941. Weather conditions were decidedly adverse in 1941. The season was dry, and later selections suffered more from drought than did earlier ones. It was anticipated that the marked differential effect on early and late selections might well obscure any relationship between yield and protein content.

1944. Growing conditions were excellent throughout the season of 1944. There was 11 in. of rainfall at Edmonton in the period May, June, and July. Crops were very heavy, and no serious setback should have been suffered by any selection of barley or wheat at Edmonton. Growing conditions at Fallis were also very good.

1945. The growing season in 1945 was dry. Only 4.7 in. of rain fell during the period May, June, and July at Edmonton. In spite of this, yields were good, and crops developed normally at both Edmonton and Fallis.

1946. Growing conditions at both Edmonton and Fallis were excellent in 1946. Lodging occurred with many selections, particularly at Edmonton, but careful handling largely eliminated errors that might have occurred in yields.

Protein Determination

All protein results were determined using the Kjeldahl method with mercuric oxide as catalyst. Protein in wheat is reported as N × 5.7 and in barley as N × 6.25. All results are expressed on the basis of 13.5% moisture.

Results

Barley

The results of all barley experiments are summarized in Tables I and II, and in Figs. 1 and 2.

TABLE I

ANALYSIS OF VARIANCE, REPRESENTATIVE BARLEY DATA

Variance due to:	Edmonton, 1945				Yield, 1946		
	D.f.	Mean squares			D.f.	Mean squares	
		Yield	Protein	Growth period		Edmonton	Fallis
Selections	99	514.06**	3.80**	146.5**	49	274.41**	336.83**
Replicates	3	4851.70**	4.83**	188.0**	3	243.07	4.62
Error	297	57.73	0.33	2.7	147	135.00	82.02

** *Exceeds the 1% point.*

The 1941 results can be dismissed with the brief comment that, despite the very adverse growing conditions, a negative correlation coefficient of

TABLE II

CORRELATION AND REGRESSION RESULTS FROM BARLEY DATA

Station and year	D.f.	Statistic		
		r_{pu}	b_{pu}, %/bu.	b_{up}, bu./%
Edmonton 1941	98	−.300**	−0.037	−2.8
1944	98	−.533**	−0.044	−6.5
1945	98	−.761**	−0.066	−8.8
1946	48	−.483**	−0.050	−4.7
Mean 1944-46	48	−.793**	−0.085	−7.4
Fallis 1946	48	−.762**	−0.091	−6.3

** *Significant beyond the 1% point.*

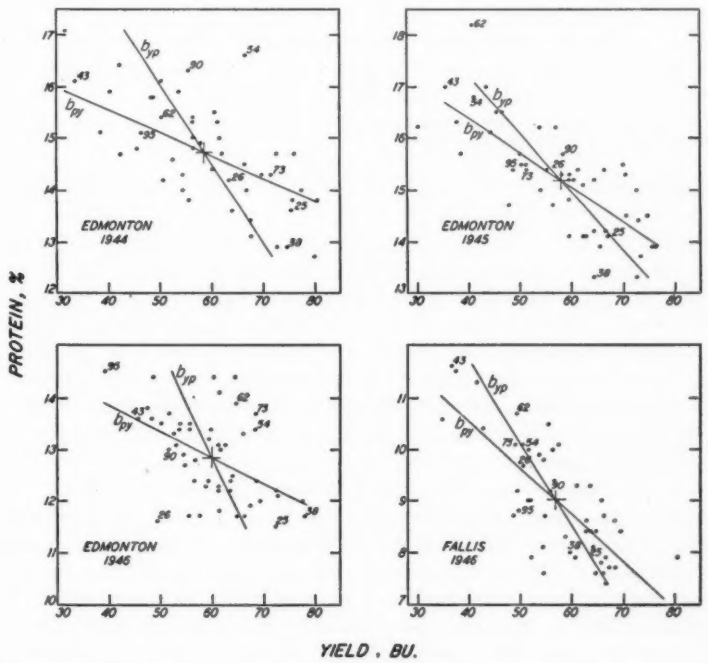

FIG. 1. *Correlation surfaces for individual tests of barley showing the interrelation between yield and protein content.*

$-$ .300** was obtained. The regression coefficient b_{py} was $-$ 0.037% per bushel, which is somewhat lower than most results obtained by other investigators. This is to be expected with the comparatively low correlation coefficient obtained.

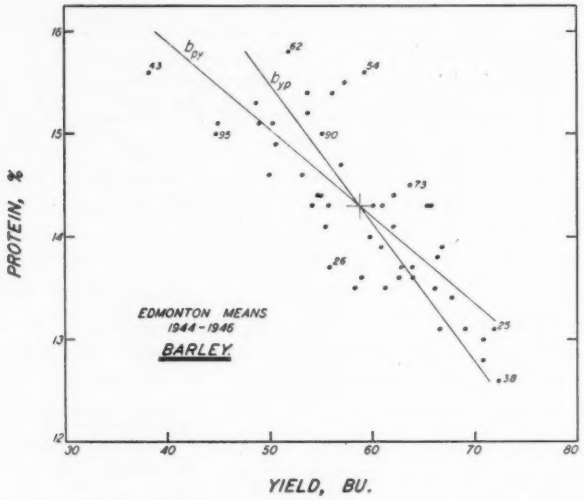

FIG. 2. *Correlation surface for the mean values from three barley tests, showing the interrelation of yield and protein content.*

Representative results of analysis of variance are given in Table I. Highly significant variation in both yield and protein content of individual strains occurred in all years. Time of maturity also varied widely in 1944 and 1945. No maturity notes were taken in 1946. The high variation in growth period was negatively associated with yield in 1944 and positively associated with yield in 1945. This difference had some effect on the numerical value of the correlation between yield and protein content but did not alter the nature or generality of the conclusions regarding this relationship. For this reason it is not considered necessary to discuss it further, except to note that it might be of some importance if practical applications of the yield–protein relationship were to be developed.

The correlation and regression coefficients are presented in Table II, while the correlation surfaces are plotted in Figs. 1 and 2. The highly significant correlation shown in every test leaves no doubt that the negative relationship between yield and protein content is the normal condition to be expected. In general, the coefficients are higher than most of those presented by other investigators and reviewed in the paper by Neatby and McCalla (3). The three-year means for results obtained at Edmonton should have the effect of

** *Throughout the paper, a highly significant correlation is marked with two asterisks, and a significant coefficient with one.*

largely eliminating environmental factors influencing the correlation. It seems definite that at least part of the association of low protein and high yield is controlled genetically, although the actual internal effects resulting in the association are almost certainly physiological in nature. This thesis could be developed but it seems advisable to leave such discussion to a later paper.

Two kinds of regression coefficients are given in Table II. Investigators interested in the general association of yield and protein content would probably plot the data as is done in Figs. 1 and 2 with yield as the independent variable. Thus one would think of variations in yield affecting the level of protein content. Perhaps, however, a much more valuable use can be made of data such as these. If yields of selections could be predicted in early stages of a breeding program it would save the plant breeder a great deal of expense and time. A protein test can be made much more cheaply and with much less material than can a yield test. It is of interest, therefore, to calculate b_{yp}, since this is the coefficient that must be used if prediction of yield is to be made.

The results in Table II show that in general a reduction of about -0.05% or more in protein content may be expected for every bushel increase in yield. The b_{py} values are usually higher with material grown on relatively infertile soils, such as occur at Fallis (3). If nitrogen supply is a limiting factor in plant development, obviously an increase in yield will mean that the nitrogen available must be distributed over more plant material, and the percentage will be lower. This is what occurs at Fallis. Despite this general change in physiological balance within the plant, the selections tend to occur in the same position on all correlation surfaces, a fact illustrated by the "tagged" values in Figs. 1 and 2.

The results for barley, therefore, substantiate earlier results. The correlation coefficients tend to be higher than in most studies, but this was expected since selection for other characters had not been carried out before the correlation studies were begun.

Wheat

The results for the wheat studies are presented in Tables III and IV and Figs. 3, 4, and 5.

The analysis of variance results show much the same relationships as did those for barley. Selections varied widely in yield and time of maturity. In one year only were protein tests made on material from individual plots, but in this one test, highly significant differences in protein content were demonstrated. As with barley, it was found that the earliness or lateness of a selection was correlated with yield, but only at Edmonton in 1945 did this association affect the correlation between yield and protein. This effect was to increase the correlation coefficient when growth period variations were eliminated. The result given in Table IV for this test is, therefore, lower than that obtained for the partial correlation ($-.638$).

TABLE III

ANALYSIS OF VARIANCE, REPRESENTATIVE WHEAT DATA

Variance due to:	Edmonton, 1945				Yield, 1946		
	D.f.	Mean squares			D.f.	Mean squares	
		Yield	Protein	Growth period		Edmonton	Fallis
Selections	99	156.36**	1.07**	25.2**	49	517.16**	302.48**
Replicates	3	1527.95**	5.59**	86.0**	3	268.19	205.40*
Error	297	61.62	0.26	5.5	147	100.76	73.83

* *Exceeds the 5% point.*
** *Exceeds the 1% point.*

TABLE IV

CORRELATION AND REGRESSION RESULTS FROM WHEAT DATA

Station and year	D.f.	Statistic		
		r_{py}	b_{py}, %/bu.	b_{yp}, bu./%
Edmonton 1944	98	−.806**	−0.053	−12.3
1945	98	−.527**	−0.048	− 5.8
1946	48	−.683**	−0.058	− 8.1
Mean	48	−.815**	−0.063	−10.6
Fallis 1944	98	−.666**	−0.091	− 4.9
1945	98	−.641**	−0.079	− 5.2
1946	48	−.762**	−0.063	− 9.2
Mean	48	−.781**	−0.073	− 8.3
Edmonton mean yield Fallis mean protein	48	−.724**	−0.048	−10.9
Fallis mean yield Edmonton mean protein	48	−.768**	−0.084	− 7.1
Edmonton–Fallis mean	48	−.872**	−0.075	−10.1

** *Significant beyond the 1% point.*

The correlation coefficients are of the same order as those obtained with barley, although on the average they are slightly higher. Since the tests were located at two stations in each of the three years, it is possible to obtain correlation and regression coefficients for entirely independent samples. When the mean yields obtained at Edmonton are correlated with the mean protein values obtained at Fallis, or vice versa, the environmental influence should be entirely eliminated. Nevertheless, the correlation coefficients for such three-year mean values are high, and show that there is an inherent tendency for a selection to combine high protein and low yield (Fig. 5). The association tends to improve as wider means are used, since the highest value

for r_{py} was obtained using the mean yield and protein values for six independent tests over a three-year period at two stations. A comparison of the correlation surfaces in Figs. 3, 4, and 5 shows this closer relationship using means.

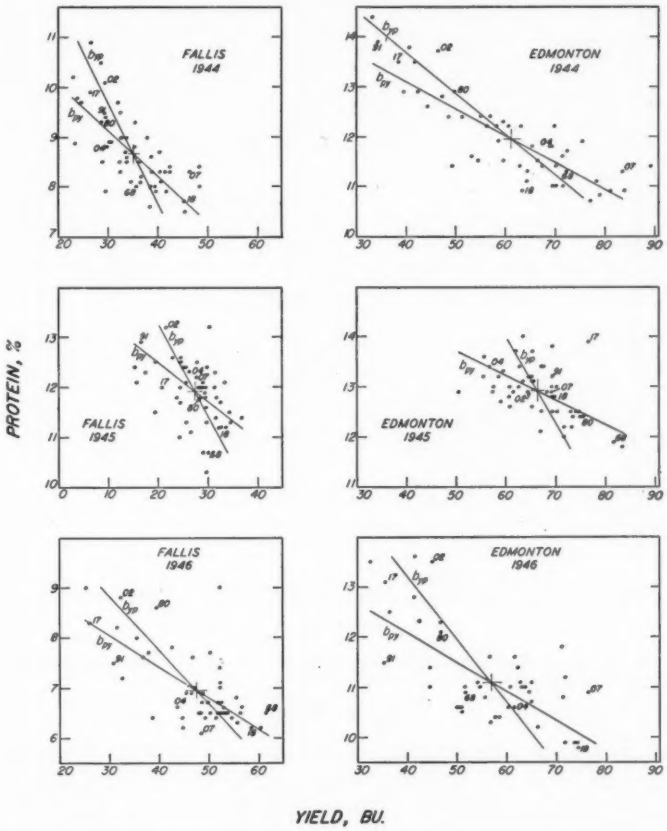

FIG. 3. *Correlation surfaces for individual tests of wheat showing interrelations of yield and protein content.*

The results in Fig. 4 are of interest in assessing the environmental influence on the yield–protein relationship. It is quite apparent that samples grown at Fallis, on low-nitrogen podsolic soil, are lower in protein and in yield than corresponding samples grown at Edmonton, on fertile black loam. The effect of the difference in environment has not changed the relative reaction of individual selections, however, as can be readily seen from the consistency with which the tagged selections retain their relative positions on the correlation surface. Thus within the limits of this study, environment affects the

general level of yield and protein content to an even greater extent than does variability among selections, but the relative effect on selections is more or less uniform. This general relationship is illustrated in a different way in Fig. 5.

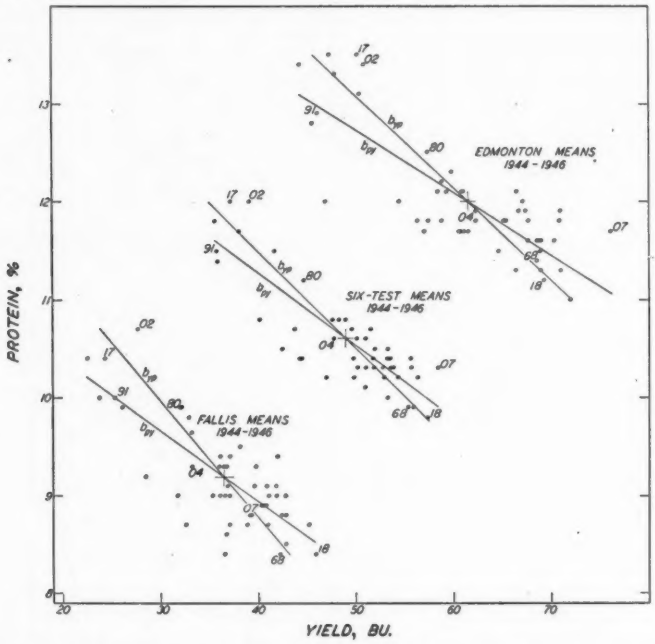

FIG. 4. *Correlation surface for mean values from six wheat tests, showing the interrelation of yield and protein content.*

Discussion

The results presented in this paper confirm the general negative relationship between yield and protein content of wheat and barley. The generally higher correlation coefficients reflect the greater suitability of random selections from single crosses for this type of study as compared with varieties in ordinary tests or selections from single crosses where elimination based on other characters has already been carried out. Even the highest correlation coefficients, however, show that a considerable part of the variability in yield or protein is not necessarily associated with the variability in the other factor. Thus it should be possible to raise the protein content of high-yielding strains, but the writers agree with Berg (1) that the extent to which this can be done is probably not great.

The most practical use to which the relationship reported in this paper could be put would be in the early elimination of poor-yielding strains. If high protein means low yield in a large majority of cases, then the strains with

the highest protein content could be eliminated before any expensive yield tests were conducted. A correlation value of − .5 is scarcely high enough to justify such elimination, but one of − .8 would certainly justify it.

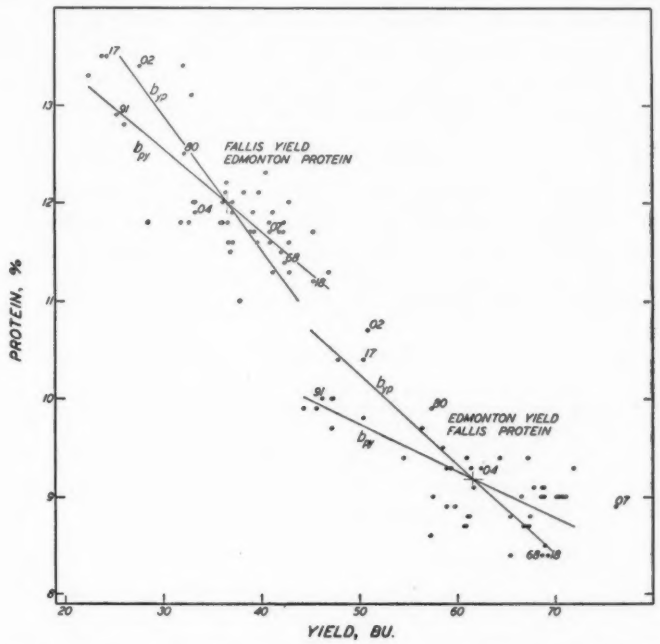

FIG. 5. *Correlation surface for mean yield values obtained at one station plotted against mean protein values obtained at a second station. This should rule out environment as a factor affecting the inherited interrelation of yield and protein content.*

Likewise a high value would be a reasonable guarantee that a large proportion of low protein selections would be high-yielding. Further discussion of the possibilities of using the relationship in this way is contained in the next paper of this series.

Reference has been made to the "inherent" tendency for yield and protein content to be negatively associated. It may be questioned whether there are specific genes for low-protein or high protein, but it seems certain that the physiological factors that determine yield and protein content are genetically controlled. The high-yielding strains apparently nearly always absorb more total nitrogen than do low-yielding strains (1),* but the percentage of protein in such strains is consistently lower. No attempt has yet been made to explain the physiological processes involved.

* *Calculations by P. F. Knowles on data obtained at Edmonton and Fallis substantiate Berg's results.*

The suggestion outlined in the introduction to this paper can now be reliably confirmed. The results obtained in the present study make it clear that it is difficult to combine high yield and high protein content in wheat and barley. If this is a desirable combination, as with hard wheat, then breeding work is rendered difficult. If, however, high protein content is not a factor in determining high quality, as with wheat for pastry flour and barley for malt, the selecting of new varieties of these crops may be speeded up by taking advantage of the relationship studied in this project.

Acknowledgments

The nature of the material used in this type of study necessitates that preliminary work be spread over a number of years. To Dr. K. W. Neatby, under whose supervision the crosses that provided selections for this study were made, and to the graduate students who assisted at various stages of the project, grateful acknowledgment is made.

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STUDIES OF CANADIAN THELEPHORACEAE

IV. *CORTICIUM ANCEPS* IN NORTH AMERICA[1]

By H. S. Jackson[2]

Abstract

A parasitic basidiomycete, referred to *Corticium anceps* (Bres. & Syd.) Gregor, has been found to be common on a large number of hosts in the Timagami Forest Reserve, Ont. The same species is known to occur also in Quebec and in four of the northern United States. The fungus is transferred to the genus *Ceratobasidium*. The history of our knowledge of this parasite is reviewed, and a description and illustration of the fungus is provided as well as a discussion of the symptomology with illustrations of the effect on several hosts. A full list of the hosts on which the fungus has so far been found in North America, with the provinces and states in which collections have been made, is given in tabular form. The incompleteness of our knowledge with reference to host specialization, infection, and colonization of the host, and overwintering of the fungus is discussed and suggestions made for future work.

Introduction

In connection with a general study of the mycological flora of the Timagami Forest Reserve in Northern Ontario, a parasitic basidiomycete which attacks a large number of hosts has been under observation for several years. The first collection was made in 1929 on *Cornus canadensis*. In 1931 and 1932 the same fungus was found on *Aralia nudicaulis* as well as on *Cornus* and was then identified as *Sclerotium deciduum* J. J. Davis. These collections were made in late August or September and were in the sterile condition. It was not until 1934 when collections were made between July 15th and August 1st that basidia were first noted. During that season the fungus was collected on 12 different hosts including *Pteridium aquilinum* var. *latiusculum*. Basidia were demonstrated on most of these hosts. The basidial fructification, as described below, is found only as a superficial film on the under side of the uninjured portions of the leaf in advance of the spreading necrotic lesions and is easily overlooked. The basidia are often absent or difficult to demonstrate in collections made later in the season but collections can be positively identified because of the presence and persistence of the characteristic infection cushions or appressorial pads, discussed later. In the fall of that year Dr. H. T. Güssow, who had visited Great Britain during the summer, called to my attention the preliminary papers by Mrs. Gregor (5, 6) dealing with a disease of the bracken fern in Scotland the causal fungus of which had been identified as *Tulasnella anceps* Bres. & Syd. and for which the combination *Corticium*

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This study was carried out, in part, with the assistance of a grant in aid of research furnished by the University of Toronto. Nos. I, II, and III of this series were published in Can. J. Research, C, 26 : 128-139 and 143-157, 1948; C, 27 : 147-156. 1949.

[2] *The writer is indebted to Miss Charlotte E. Dill for the preparation of the drawings for the text figure, to Miss E. Ruth Dearden for assistance in the preparation of the manuscript, to Dr. J. H. Soper for checking the identification of most of the hosts, and to Dr. S. M. Pady for the privilege of examining recent collections made in Quebec.*

anceps (Bres. & Syd.) Gregor was provided. Comparison of our material with Mrs. Gregor's description and with the type distribution of *Tulasnella anceps* (Sydow, Myc. germ. 858) left little doubt that our fungus should be referred to that species. In the meantime Mrs. Gregor's more detailed paper (7) on the same subject was published. Her extensive observations on the symptoms and progress of the disease, and on the morphology of the fungus as it occurs on bracken fern and the characteristics of the growth in culture, correspond so exactly with our own that there is little question that we had been concerned with the same species.

History

Tulasnella anceps was originally described by Bresadola & Sydow (13, p. 490) on *Pteridium aquilinum*. No other host had been recorded for the fungus in Europe until Mrs. Gregor (7) reported its occurrence in nature on *Aspidium Filix-mas* in Scotland. In connection with her study of the disease on the bracken fern, however, Mrs. Gregor conducted a series of infection experiments in the course of which successful transfer of the fungus from bracken was obtained on eight other fern hosts, namely: *Aspidium Filix-mas*, *A. spinulosum*, *A. aculeatum* var. *lobatum*, *Asplenium Trichomanes*, *Polypodium vulgare*, *Blechnum spicant*, *Cystopteris fragilis*, and *Scolopendrium vulgare*.

Whether or not any name has ever been applied to the sterile condition of the fungus in Europe we have been unable to determine. In America, however, the late Dr. J. J. Davis (4) described *Sclerotium deciduum* from Wisconsin recording it on a number of hosts including both pteridophytes and angiosperms. Duplicates of several of these collections made by Dr. Davis were in the herbarium of the University of Toronto and an examination of these left no doubt that the fungus he described in the sterile condition and the one we have had under observation are the same. The first host listed by Davis in connection with the original description of *Sclerotium deciduum* was *Adiantum pedatum*. A duplicate in the herbarium of the University of Toronto, of a collection on that host made by Davis at Devil's Lake, Wis., Aug. 5, 1913, was examined and the basidia of *Corticium anceps* are present in beautiful condition. More recently, through the courtesy of Dr. E. M. Gilbert, all the collections of *Sclerotium deciduum* Davis which were then in the herbarium of the University of Wisconsin were examined for basidia. A total of 47 collections occurring on 17 hosts (see Table I) were included. Basidia were demonstrated on 17 of these collections occurring on nine hosts as follows: *Pteridium aquilinum* var. *latiusculum*, *Adiantum pedatum*, *Onoclea sensibilis*, *Pteretis pennsylvanica* (= *Onoclea struthiopteris*), *Aralia nudicaulis*, *Diervilla Lonicera*, *Hydrophyllum virginianum*, *Solidago serotina*, and *Lysimachia ciliata*. The other Wisconsin collections were made too late in the season to show the basidia or were made expressly to illustrate the sclerotial condition.

Relationship

Corticium anceps as I have observed it and as described by Mrs. Gregor is quite certainly distinct from *Pellicularia filamentosa* (Pat.) Rogers (= *Corticium Solani* (Prill. & Del.) B. & G.). While both species are parasitic, *C. Solani* ordinarily attacks the underground parts of the host or the stem near the surface of the ground and the basidial fructification is ordinarily found at the base of the stem or petiole, only occasionally spreading out on the leaves of low-growing hosts. In *C. anceps*, on the contrary, the leaves or pinnae of the host are attacked directly. It has never been observed fruiting at the base of the stem. Then too the mycelium of *C. Solani* is of greater diameter than in *C. anceps* and does not develop the minute appressorial pads on the host or in culture which are so characteristic of the latter.

Corticium anceps, because of the character of the basidium and the habit of spore germination by repetition, belongs in the genus *Ceratobasidium* Rogers. This genus was erected by Rogers (11) to include a group of "delicate corticioid" forms, the older species of which had been included in *Corticium*, "whose holobasidia show the division into hypo- and epibasidia and whose spores" germinate by repetition. Whether or not one accepts *in toto*, Rogers' (10) interpretation of the basidial morphology in these groups, it is evident from a study of the forms included in *Ceratobasidium* that they represent a natural group of species. The species of *Ceratobasidium* possess characters which suggest relationship with the Heterobasidiomycetes, through the Tulasnellaceae on the one hand, and to the Homobasidiomycetes through *Pellicularia* (= *Botryobasidium* Donk.) on the other. In 1944, Martin (8) included *Ceratobasidium* in the Tulasnellaceae of the Tremellales. In a more recent paper Martin (9) has erected the family Ceratobasidiaceae to include the genus *Ceratobasidium*, placing it at the base of the Tremellales. He also suggests that *Pellicularia* and possibly related forms should sometime form the basis for a co-ordinate family at the base of the Homobasidiomycete series. In the opinion of the writer *Ceratobasidium* is too closely related to *Pellicularia*, as reviewed by Rogers (12), to justify such a wide separation in the classification of Basidiomycetes. It would seem more logical to include *Pellicularia* in the family Ceratobasidiaceae and place that family at the base of the Homobasidiomycete series. The two genera approach each other very closely, especially through the parasitic species.

The Fungus

At present the writer is inclined to accept the genus *Ceratobasidium* as delimited by Rogers and to transfer to it the species under discussion:

Ceratobasidium anceps (Bres. & Syd.) comb. nov.

Tulasnella anceps Bres. & Syd., Ann. Mycol. 8 : 490. 1910.

Sclerotium deciduum J. J. Davis, Trans. Wisc. Acad. Sci. 19 : 689. 1919.

Corticium anceps (Bres. & Syd.) Gregor, Ann. Mycol. 30 : 464. 1932.

Basidial fructification occurring as a delicate, closely appressed, often almost invisible film or weft of hyphae spreading over the apparently uninjured lower surface of the leaves of the host, usually observed as occurring at the margin or in advance of spreading necrotic lesions; sometimes developing sufficiently to form a delicate separable pellicle. The film or pellicle made up of intertwining, branching, thin-walled, simple-septate hyphae 3.5-5.5 μ in diameter, branching often at right angles. Basidia short cylindric, broadly clavate, obovate, or irregular, 10-18 $\times$ 8-12 μ, often formed directly from hyphal cells when they may have a truncate base drawn out at either side or to one side if formed terminally. "Epibasidia", usually four, occasionally three, developing at first as subglobose projections, becoming cylindric and finally terete, arcuate, or rather widely divergent, 10-16 μ long. Basidiospores asymmetrically ellipsoid, broadest below the middle, flattened and appearing straight on one side, with prominent lateral apiculus, varying somewhat in size on different hosts, 9-13 $\times$ 4.5-7 μ, germinating by repetition; walls thin, smooth, nonamyloid (Fig. 1).

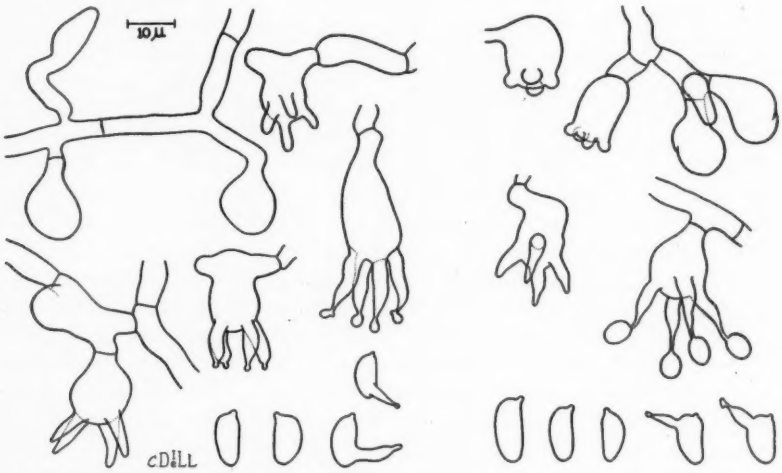


FIG. 1. *Ceratobasidium anceps, hyphae, basidia, and spores. The group at left from Aralia nudicaulis, at the right from Pteridium aquilinum var. latiusculum.*

Following the advancing basidial fructification and often beginning to appear before basidial production has ceased, the hyphae become aggregated at close intervals into what may be termed appressorial pads or infection cushions (Gregor). As these develop the hyphae intertwine closely with septa at frequent intervals. At maturity a compact palisadelike layer of parallel hyphal tips develops on the side next the host epidermis with looser intertwined threads on the free side. Necrotic tissue develops as the cushions

mature and the latter persist on the dead areas as brownish specks and furnish a positive means of identification after the basidial fructification has disappeared.

Finally white fluffy masses of mycelium are developed on the surface of the dead areas at irregular intervals and these are gradually transformed into the characteristic brown sclerotia which, when fully developed, are deciduous.

Care must be taken not to confuse *C. anceps* with another related form which is not uncommon. One occasionally finds a *Corticium* in fruiting condition on the bases of stems of living ferns or of angiosperms. Sometimes this fungus may form a very delicate whitish film of mycelium and basidia not only on the stems or petioles but extending over considerable areas of the under surface of the living leaves. There is however in such cases no evidence of parasitism. The fungus is apparently growing superficially over the host, presumably spreading as a saprophyte from surrounding humus. No mycelial pads or sclerotia such as occur in *C. anceps* are formed and the host appears not to be affected. We have collections of this form from Bear Island, Lake Timagami, on the bases of stems of *Pteridium aquilinum* var. *latiusculum*, *Dryopteris Phegopteris*, *Onoclea sensibilis*, *Aralia nudicaulis*, *Diervilla Lonicera*, *Aster macrophyllus*, seedling *Fraxinus nigra*, and on stems and leaves of *Viola* sp., *Rubus pubescens*, and on an unidentified grass. A collection of the same form was also made at Inlet, New York, in 1934 on petioles and leaves of *Rubus pubescens* and on rachis and fronds of *Onoclea sensibilis*. While showing some variation in spore size and diameter of mycelium these collections are all referred, for the present, to *Ceratobasidium cornigerum* (Bourd.) Rogers (= *Corticium cornigerum* Bourd.) which, as at present interpreted, is also commonly found as a saprophyte on dead bark and wood of various trees. This species does not form sclerotia or appressorial pads in culture. It is quite different from *C. Solani* though fruiting in a similar relation to the host and has perhaps sometimes been confused with that species. Mrs. Gregor (7, p. 403) refers briefly to another *Corticium* which she found at the base of bracken fronds in connection with her studies of *C. anceps*, which appeared to be quite harmless. It is possible that this may also have been *C. cornigerum*, though no description was furnished, and the species was not identified.

Hosts and Distribution

Our own collections in Ontario have been made entirely in the Timagami Forest Reserve. Most of these collections have been made in a small area about 50 yd. long along a trail on Bear Island. The fungus was also observed in abundance in the vicinity of Duchesnay, Que., at the time of the Mycological Society Foray held in late August 1938. It was particularly abundant there on *Dennstaedtia punctilobula* but present, for the most part, in the late sclerotial phase. Collections were made on seven hosts and had the observations been made a month earlier no doubt many other hosts could have been found. More recently other collections from Quebec have been made by Dr. S. M. Pady in the vicinity of Lennoxville and near Nemaygo. A number

of collections were made by the late Prof. H. H. Whetzel and students in Maine, New Hampshire, and New York. These collections, together with those made by Dr. Davis in Wisconsin discussed previously, form the basis of the host list in Table I.

TABLE I

HOSTS AND DISTRIBUTION*

Pteridophytes		Dicotyledons—*concluded*	
Adiantum pedatum L.	W, Q	*Hydrophyllum canadense* L.	NY
Athyrium Filix-femina (L.) Roth var. *Michauxii* (Spreng.) Farwell	O	*Hydrophyllum virginianum* L.	W, NY
Cystopteris bulbifera (L.) Bernh.	Q	*Lactuca biennis* (Moench) Fern.	O
Dennstaedtia punctilobula (Michx.) Moore	Q, M	*Linnaea borealis* L. var. *americana* (Forbes) Rehd.	O
Dryopteris disjuncta (Rupr.) Morton	O	*Lonicera canadensis* Marsh. ?	O
Dryopteris Phegopteris (L.) C. Chr.	O	*Lycopus americanus* Muhl.	O
Dryopteris spinulosa (O. F. Muell.) Watt.	O, Q	*Lysimachia ciliata* L.	W
Dryopteris Thelypteris (L.) Gray var. *pubescens* (Lawson) Prince	NH	*Mitella diphylla* L.	W
Onoclea sensibilis L.	O, W, NY	*Mitella nuda* L.	O
Pteretis pennsylvanica (Willd.) Fern.	O, W	*Petasites palmatus* (Ait.) Gray	O
Pteridium aquilinum (L.) Kuhn var. *latiusculum* (Desv.) Underw.	O, Q, W	*Plantago major* L.	O
		Prenanthes sp.	Q
Dicotyledons		*Prunella vulgaris* L.	O
Acer spicatum Lam.	O	*Pyrola elliptica* Nutt. ?	O
Acer sp. (seedling)	Q	*Ranunculus septentrionalis* Poir.	W
Achillea Millefolium L.	O	*Ranunculus acris* L. ?	O
Apocynum androsaemifolium L.	O	*Ribes glandulosum* Grauer	O
Aralia nudicaulis L.	O, Q, W, NY	*Rubus allegheniensis* Porter	W
Aster macrophyllus L.	O	*Rubus strigosus* Michx.	O, Q
Aster sp.	W	*Rubus pubescens* Raf.	O
Bidens frondosa L.	W	*Silphium terebinthinaceum* Jacq.	W
Chrysanthemum Leucanthemum L.	O	*Solidago canadensis* L.	W
Cornus canadensis L.	O, Q, NH	*Solidago gigantea* Ait. var. *leiophylla* Fern.	W
Corylus cornuta Marsh.	O	*Taraxacum officinale* Weber	O
Dicentra Cucullaria (L.) Bernh.	Q	*Tiarella cordifolia* L.	Q
Diervilla Lonicera Mill.	O, W	*Trientalis borealis* Raf.	O, W, Q
Epilobium angustifolium L.	O	*Viburnum alnifolium* Marsh	Q
Epilobium glandulosum Lehm. var. *adenocaulon* (Haussk.) Fern.	O	*Viola* sp.	O
Fragaria vesca L. var. *americana* Porter	O	Monocotyledons	
Fragaria virginiana Duch.	W	*Carex arctata* Boot. ?	O
Galium triflorum Michx.	O	*Clintonia borealis* (Ait.) Raf.	O, Q
Hackelia virginiana (L.) I. M. Johnston	O	*Habenaria viridis* (L.) R. Br. var. *bracteata* (Muhl.) Gray	O
Hieracium scabrum Michx. ?	Q	*Maianthemum canadense* Desf.	O, Q
		Streptopus roseus Michx.	O
		Trillium cernuum L.	O
		Trillium sp.	Q

* *O = Ontario, Q = Quebec, M = Maine, NH = New Hampshire, NY = New York, W = Wisconsin. All collections on which the above list is based are represented in the mycological herbarium of the University of Toronto, TRT.*

Symptomology

Mrs. Gregor (7, pp. 403-406) has given an excellent account of the development of the infected areas and of the symptoms of the disease as it occurs on bracken. Our own observations on ferns correspond very closely with her account. In general the ultimate effect of infection is quite similar on the different species. Older infected areas of the pinnae become brittle and

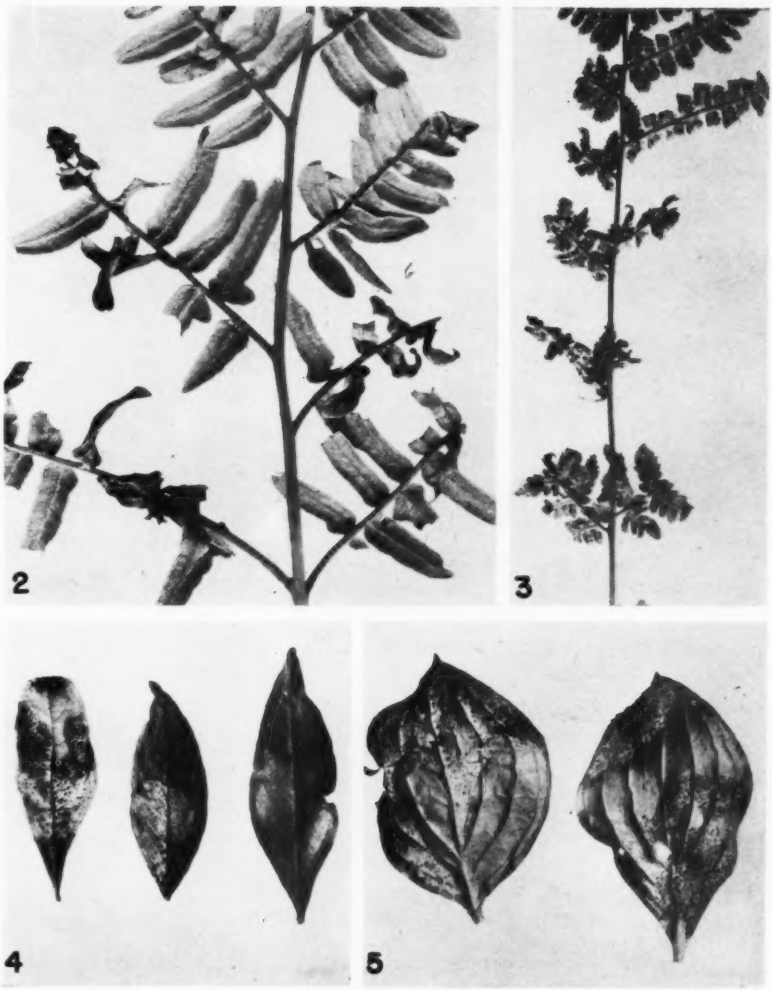
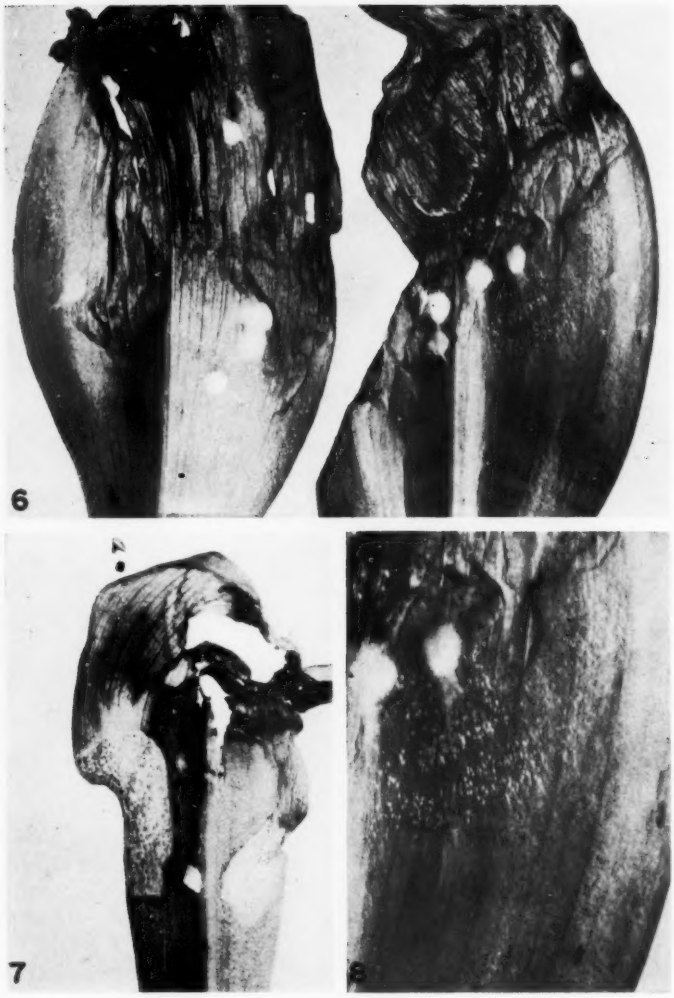


FIG. 2. *Result of infection by Ceratobasidium anceps on Pteridium aquilinum var. latiusculum. Natural size.*

FIG. 3. *Same, on Dryopteris spinulosa.*

FIG. 4. *Same, on Trientalis borealis, the two leaflets at left show the under surface, the one at right the upper surface. Note appressorial pads on lesions at left.*

FIG. 5. *Same, on Cornus canadensis, lower surface showing lesions with appressorial pads.*



FIGS. 6, 7, 8. *Infection of Ceratobasidium anceps on Clintonia borealis, proceeding from tip of leaf, downward. Note appressorial pads and sclerotia in Fig. 6. Young and mature appressorial pads are shown in Fig. 7. Fig. 8 is an enlargement × 2 of a portion of the leaf at right of Fig. 6 and shows young appressorial pads. The basidial fructification is present below the pads on the uninjured leaf surface.*

usually tend to drop away leaving a more or less naked rachis. This was particularly noted in the case of *Dennstaedtia punctilobula* in the vicinity of Duschesnay, Que. In one locality, in a moist low woods, infection by *C. anceps* had reduced the leafy portions of the fronds almost completely over a considerable area. Infected fronds of *Pteridium aquilinum* var. *latiusculum* and of *Dryopteris spinulosa* are shown in Figs. 2 and 3.

On the angiosperms the gross symptoms are very variable on the different species attacked. The necrotic areas vary in size, shape, and coloring, though some shade of brown is predominant. Illustrations (Figs. 4 to 10) of the effect of the fungus on several hosts will be found in Plates I to III and the following account deals primarily with those examples.

On *Clintonia borealis* (Figs. 6, 7, 8), infections are frequently at the tip of the leaf, proceeding downwards. In advance of necrosis, an inconspicuous basidial phase may be perceived with the aid of a lens as a delicate pelliculose film on the lower surface of the leaf. Appressorial pads are first visible beyond the necrotic margin as minute white thickenings of the surface mycelium (Fig. 8), which at maturity are dark brown and thickly scattered over the injured portion of the leaf (Fig. 7). Sclerotia, beginning as fluffy masses of hyphae, are relatively abundant. The infected tissue becomes discolored, finally brown, a blackish green, or almost colorless and may become brittle and drop away.

On *Aralia nudicaulis* (Figs. 9, 10), lesions are diffuse, finally involving all green tissue of an infected leaf. The infections are irregularly spreading, on the lower surface conspicuously punctate from the appressorial pads. The advancing margin of necrosis is sharply delimited, and often a dark sepia brown. Behind the margin, the killed tissue may be variously colored, fading rapidly to a buff or olive green, ultimately becoming straw colored or almost colorless, the upper surface more intensely colored than the lower. The gross appearance of heavily infected leaves varies considerably. Many are marked by large spreading islands of necrotic tissue, each sharply delimited and separated one from the other by bands of green tissue; many others have one necrotic area merging with another, the whole a mosaic of discolored, mottled, dead tissue.

On *Trientalis americana* (Fig. 4), infected areas are frequently reddish brown and sufficiently thin and transparent that the appressorial pads are as conspicuous from the upper surface as from the lower. Young infections are spotty, orbicular, but spreading and eventually involving the entire leaf. The apparently uninjured tissue of infected leaves may turn reddish. This same reaction has been observed on collections of *Cornus canadensis* (Fig. 5) made late in August and September. This may be a seasonal variation co-related with the maturation of the host, for July collections do not show the same discoloration. Lesions on *Cornus* are sharply differentiated, brown or buff in the center, becoming white, and surrounded on the upper surface by a narrow purplish rim. Leaves of late collections may be entirely reddish purple in advance of necrosis.

On *Maianthemum canadense* and *Apocynum androsaemifolium* much of the green tissue becomes chlorotic in advance of spreading necrosis; in *Acer spicatum*, *Clintonia borealis*, and *Diervilla Lonicera* chlorosis is less evident. This variation in symptomology has been described on the basis of dried herbarium specimens collected over several seasons. Controlled infection experiments would provide valuable data on the progress of the disease and the reaction to the disease by selected hosts. However, the presence of appressorial pads on necrotic lesions of a host is apparently indisputable evidence of the parasitic phase of this fungus.

Cultures

Pure cultures are readily obtained from germinating basidiospores or by direct transfer of the appressorial pads to poured agar plates. In the latter case subtransfers from the margin of the advancing colony will give pure cultures, though often the original transfer appears to be pure. We have not made any extensive study of cultures but have verified most of the observations recorded by Mrs. Gregor (7). Cultures obtained from the fungus on different hosts do not vary appreciably so far as we have observed them. In Fig. 12 a photograph of a Petri dish is shown in which colonies originating from cultures obtained from six different hosts were grown side by side. The central colony originated from *Pteridium aquilinum* var. *latiusculum*. Starting at the top and reading clockwise the outer ring of colonies originated from *Clintonia borealis*, *Taraxacum officinale*, *Dryopteris spinulosa*, *Clintonia borealis*, *Cornus canadensis*, and *Streptopus roseus*. No appreciable difference in these colonies was noted at this stage of growth or later. In Fig. 11, two plates are shown in which the fungus has been allowed to grow until the whole plate is covered. The upper one originated from a culture obtained from *Streptopus roseus* and the lower one from *Pteridium aquilinum* var. *latiusculum*. The latter culture compares very favorably with Mrs. Gregor's Fig. 10 except that our culture shows more evident zonation. This difference may have been due to the conditions under which the plates were handled during the period of growth. More sclerotia were formed in the culture from the *Pteridium* as compared with the one from *Streptopus* but, as suggested by Mrs. Gregor, such a difference may have been due to the depth of the medium. Appressorial pads developed abundantly on the sides of the culture dishes or tubes as noted by Mrs. Gregor in her cultural studies.

No basidia have been observed to form in culture either by Mrs. Gregor or the writer. It seems probable that they would develop in polysporous cultures

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FIGS. 9 and 10. *Infection of Ceratobasidium anceps on Aralia nudicaulis to illustrate the extensive lesions. Fig. 9 shows the upper surface, Fig. 10 the lower. Note mottled effect on the upper surface.*

FIG. 11. *Cultures of Ceratobasidium anceps. The upper plate from Streptopus roseus, the lower one from Pteridium aquilinum var. latiusculum.*

FIG. 12. *Plate with colonies of Ceratobasidium anceps obtained from six different hosts. See text.*

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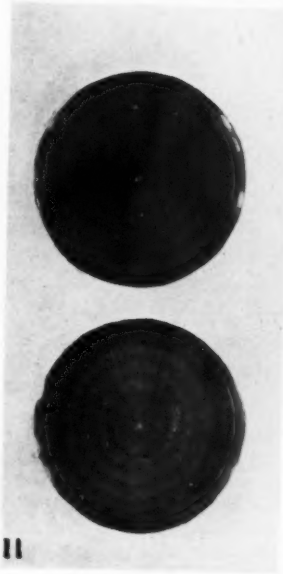

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or in cultures from appressorial pads or sclerotia if the proper conditions were provided. Whether they would develop in cultures obtained from single basidiospores might depend on whether or not the fungus is homothallic or heterothallic, a problem which has not yet been investigated.

Discussion

The primary purpose of the present contribution has been to record the occurrence, host range, and distribution of *Corticium anceps* in North America. Only preliminary observations have been made with reference to other intriguing problems which have become obvious from our admittedly incomplete study of this very interesting parasite. It seems desirable, however, to review these problems briefly and present what is known about them as a basis for future study.

The fact that the fungus is recorded in Europe and Great Britain only on ferns while our observations indicate a wide host range, immediately raises the question of the possibility of the existence of host specialized forms or races. Is the form studied by Mrs. Gregor more restricted in host range than the one occurring at Bear Island, Timagami, where our own observations have been made? Is there more than one strain present in the latter region? Will isolates of the fungus obtained in other North American localities prove comparable in host relations to those to be obtained in the Bear Island area? The fact that the fungus has not been found on other hosts than ferns in Europe and Great Britain is suggestive that the race occurring there is more restricted in host range than the one or more races occurring in N. America. On the other hand it is possible that infections on angiosperms have been overlooked. Mrs. Gregor (7), as noted above, in controlled inoculation experiments, was able to transfer the fungus from bracken to eight other fern hosts. Unfortunately she attempted inoculation on only two species of angiosperms, both exotic species—*Solanum tuberosum* and *Lycopersicon esculentum*—and did not attempt inoculation on any native plants. The two solanaceous hosts were selected because of the assumption that *C. anceps* was closely related to *Corticium Solani* (Prill. & Del.) Bourd. & Galz. and presumably also because Bourdot and Galzin (1, p. 242) had confused *Tulasnella anceps* with *C. vagum* Berk. & Curt. with which species Burt (2, 3) had included *C. Solani*. These inoculations gave negative results.

As noted in the introduction, our first observations of the disease were made on angiosperms. A summary of our collections to date shows that in a relatively small area on Bear Island in Lake Timagami the fungus has been found on a total of 41 hosts, including 7 species of pteridophytes, 6 monocotyledons, and 28 dicotyledons (see Table 1, p. 246). No entirely satisfactory controlled inoculation experiments have yet been conducted. However, in May 1947 a preliminary series of inoculations was made by Miss E. Ruth Dearden in the greenhouse at the Department of Botany, Toronto, using cultures obtained at Bear Island, Timagami, in the summer of 1946. From a culture obtained from the fungus on *Pteridium aquilinum* var. *latiusculum*,

infection with incipient necrotic lesions and the development of appressorial pads was obtained on cultivated bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*), and on an exotic species of *Pteris*. Spreading of the fungus from the inoculum with the development of appressorial pads was obtained on corn (*Zea Mays*) but no necrotic lesion developed. From a culture isolated from *Aralia nudicaulis*, incipient necrotic lesions with the development of appressorial pads was obtained on the *Pteris*, bean, tomato, and corn. Basidia were not developed in any of these experiments. The conditions necessary for infection and the normal development of the basidial fructification and of typical lesions are evidently rather exacting and were not duplicated with entire success. Field inoculations, not controlled or protected, made by applying diseased foliage to apparently healthy leaves of other hosts have been made as follows: from *Aralia nudicaulis* to *Pteridium aquilinum* var. *latiusculum;* from the latter to *Clintonia borealis* and to *Rubus strigosus*. These preliminary inoculation experiments are not interpreted as conclusive but seem to indicate that the fungus occurring at Bear Island may be transferable from ferns to angiosperms and vice versa.

The writer has inclined to the view, based primarily on field observations over a period of 14 years, that the fungus in the Bear Island area is primarily of one strain. The chief basis for this view, inconclusively supported by the preliminary inoculation experiments mentioned above, has been the occurrence of the fungus on a wide host range in a relatively small area, together with the fact that infections of the fungus have frequently been observed in the field passing from one host to another where the foliage is in contact. Following is a list of such cases that have been observed and of which specimens have been preserved. The infection appeared to be progressing from the one host to the other as indicated:

from *Dryopteris spinulosa* to *Lactuca spicata*
from *Dryopteris disjuncta* to *Ribes prostratum*
from *Pteretis pennsylvanica* to *Aralia nudicaulis*
from *Aralia nudicaulis* to *Corylus rostrata*
from *Aralia nudicaulis* to *Petasites palmatus*
from *Cornus canadensis* to *Corylus rostrata.*

Controlled inoculations on a wide variety of native plants using cultures from various hosts and localities in North America should ultimately be made in comparison with cultures obtained from bracken in Scotland. Until this is done the problem of the existence of host specialized races cannot be finally answered.

The sequence of events as observed in connection with the development of the mature lesions is suggestive of an interesting type of parasitism. As noted above, the basidial fructification appears to develop for the most part on the apparently uninjured surfaces of the leaves. It is easily stripped off as a thin film and no evidence is available that it forms any direct contact with the epidermal cells of the host. In the case of older infections this film

is apparently dependent for its development primarily on food available from the hyphae in the necrotic lesions though there is a possibility that substances associated with the outer layers of the cuticle may furnish some sustenance. Initial local infections previous to the development of necrotic lesions present a special problem which requires further investigation. In August and later basidia are seldom found but an advancing film of sterile hyphae may be present in actively spreading infections.

From a study of microtome sections and of whole mounts cleared in lactophenol and stained with cotton blue, no evidence was obtained of the presence of mycelium within the leaf beneath the advancing basidia bearing hyphae, until the appressorial pads begin to develop. The presence of intercellular hyphae in the tissues can be observed soon after the young appressorial pads are recognizable. On present evidence these structures appear to be directly related to the colonization of the tissues by the fungus. As has been stated by Mrs. Gregor (7), direct penetration by hyphae arising from the underside of the appressorial pads can readily be demonstrated in microtome sections. Further study is needed to determine whether or not infection may also occur through stomates.

There are several aspects of the general biology of the fungus which have not been fully determined. The sclerotia which ultimately form in connection with the host lesions and which develop abundantly in culture quite certainly perform an important function in the life cycle. These sclerotia are deciduous when fully formed and drop to the ground. Cultures of the fungus may be readily obtained from sclerotia developed in culture or on the host. Mrs. Gregor (7) has shown that sclerotia are viable after overwintering on the bracken and hence are capable of carrying the fungus over the winter.

The further history to the time of initial infection is unknown. Do the sclerotia develop to a saprophytic growth with production of basidia from which air-borne spores are disseminated and cause the initial infections directly on the pinnae or leaves of the host or does infection first occur directly from sterile mycelium as the fern fronds or the early growth of perennial angiosperms push through the ground in early spring? There would seem to be little doubt that the basidiospores are capable of spreading infection from plant to plant and perhaps from host to host during the early summer, but this problem needs further study.

Is the sole function of the appressorial pads that which is associated with the colonization of the tissues by the fungus or may they also function as microsclerotia and serve as a second means of carrying the fungus over from season to season?

The nuclear history of the fungus should ultimately be investigated and should be studied from cultures obtained from monospores as well as from polyspores or from the appressorial pads or sclerotia. The hyphae in the host tissue appear to be dikaryotic so far as observed.

Since clamps are not formed at the septa the usual method of determining whether the fungus is homo- or heterothallic is not available. The development of a method of obtaining consistent production of basidia in culture would be of great assistance in connection with the elucidation of this problem. If the species proves to be homothallic, where does the dikaryon arise?

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THE INTERMEDIATE METABOLISM OF *PSEUDOMONAS AERUGINOSA*

III. THE APPLICATION OF PAPER CHROMATOGRAPHY TO THE IDENTIFICATION OF GLUCONIC AND 2-KETOGLUCONIC ACIDS, INTERMEDIATES IN GLUCOSE OXIDATION[1]

By Flora C. Norris[2] and Jack J. R. Campbell[3]

Abstract

The technique of paper chromatography has been adapted to the identification of gluconic, 2-ketogluconic, and α-ketoglutaric acids. Combinations of methyl and ethyl alcohol were found to be the most suitable solvents and ammoniacal silver nitrate was found to give the most satisfactory reaction. When grown under normal physiological conditions where glucose was metabolized to carbon dioxide and water, *Pseudomonas aeruginosa 9027* was shown to have oxidized glucose by way of gluconic and 2-ketogluconic acids. Since a strong system for oxidizing both gluconic and 2-ketogluconic acids was demonstrated, the presence of these acids over at least an eight hour period of growth is taken as evidence of their importance as intermediates in the oxidation of glucose by this organism.

The meager information available on the intermediate products formed during the degradation of glucose by oxidative organisms such as *Pseudomonas aeruginosa* has been obtained under rigorous conditions usually on a commercial scale and the data available from these studies are thus not necessarily valid under normal physiological conditions. Pervozvanskii (9) has reported high yields of gluconic and ketogluconic acids during glucose oxidation by fluorescing bacteria when conditions of intense aeration and heavy concentrations of substrate were employed. In similar investigations Lockwood, Tabenkin, and Ward (5) have compared a number of cultures of *Phytomonas* and *Pseudomonas* with respect to their abilities to produce gluconic and 2-ketogluconic acids. Yields of 58 to 96% gluconic acid and greater than 70% 2-ketogluconic acid were obtained under commercial conditions although only traces of these compounds were produced under conditions in which aeration was minimal. *Pseudomonas aeruginosa 9027*, the organism used in the present work, has also been shown to produce appreciable quantities of 2-ketogluconic acid from glucose under conditions of intense aeration (6). Lockwood and Stodola (4) demonstrated another possible step in the dissimilation of glucose when they isolated α-ketoglutaric acid during submerged fermentations of glucose, gluconate, or 2-ketogluconate by *Pseudomonas fluorescens*. Under the conditions of these experiments, however, the acids appear as end products and although they indicate a mechanism available to the cells they are not necessarily intermediate products in the normal oxidation of glucose to carbon dioxide and water.

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A study of the intermediate metabolism of an oxidative organism such as *P. aeruginosa* presents many difficulties. The organism oxidizes its substrate rapidly, and in general completely, to carbon dioxide and water, and, because of this strong oxidative power, under normal physiological conditions few of the intermediate compounds accumulate in sufficient quantity to be isolated and identified in the usual manner. In dealing with an organism about which so little is known, it did not seem justifiable to resort to the use of specific enzyme inhibitors or aeration as means of bringing about the accumulation of incompletely oxidized products. The technique of simultaneous adaptation (10), which appeared to be a useful tool for studying this organism, has been found to have serious limitations (1). Consequently it was necessary to employ a procedure that would separate and identify closely related compounds when present in only trace amounts. The technique of paper chromatography (2) suggested itself in this connection.

The purpose of the present study was to apply the technique of paper chromatography to the identification of the initial intermediate compounds formed when glucose is dissimilated by *P. aeruginosa* under normal physiological conditions.

Methods

The culture of *P. aeruginosa ATC 9027* employed was a typical strongly pigmenting strain. Stocks were maintained on liver gelatin agar and refrigerated after growth had been initiated at 30° C. Medium for growth of the organism was prepared as follows: ammonium dihydrogen phosphate 0.3%, magnesium sulphate septahydrate 0.1%, and iron 0.5 p.p.m. (as ferrous sulphate) were adjusted to pH 7.2, dispensed in 100 ml. quantities in Roux flasks, and sterilized by autoclaving at 15 lb. pressure for 15 min. Before flasks were inoculated, glucose that had been sterilized through sintered glass as a 50% solution was added aseptically to a final concentration of 0.5% and dipotassium hydrogen phosphate that had been sterilized by autoclaving as a 10% solution was added to a final concentration of 0.3%. Inoculum consisted of 1.0% of a 24 hr. culture grown in glucose ammonium phosphate medium. After subculture from the refrigerated stock the culture was always transferred at least twice at 24-hr. intervals in glucose ammonium phosphate medium before being used as inoculum.

For the preparation of concentrated supernatants, the organism was grown in 200 ml. of glucose ammonium phosphate medium at 30° C. for either 16 or 24 hr. before the cells were removed by centrifugation. The clear supernatant was adjusted to pH 7.2 with 4 *N* sodium hydroxide and then heated in a boiling water bath for five minutes to aid in suppressing further enzyme action. The supernatant was cooled and immediately vacuum distilled under nitrogen at approximately 30° C. until the final volume was 20 ml. Since the organism has been shown to be a strict aerobe (6) this method of concentrating under nitrogen was employed in order to prevent further dissimilation of glucose during the bubbling necessarily accompanying vacuum distillation.

A control flask containing 200 ml. of uninoculated medium was treated in a similar manner.

Paper chromatography was carried out both by the more conventional descending method (2) and by the ascending method (11). The latter method was used in exploratory work while the former was used in the subsequent more exact determinations. In the ascending chromatograms the compounds being tested were introduced as drops from a Pasteur pipette; these drops were placed at intervals 1½ in. from the bottom of a sheet of Whatman No. 1 filter paper which had been cut to measure 13 in. × 22 in. The sheet was pinned in the form of a cylinder and set in a large Petri dish containing 25 ml. of solvent. The cylinder and dish were placed in a clean wastebasket, a wooden cover was put in place and the sheet was irrigated at room temperature for 15 to 18 hr. The sheet was then dried and sprayed with 0.1 *N* silver nitrate in 5 *N* ammonium hydroxide. The sprayed sheet was allowed to dry in the absence of direct light. In the descending chromatograms, drops of the two supernatants, the concentrated medium and known compounds (2 mgm. per ml.) were introduced near the top of a sheet of Whatman No. 1 (18 in × 22 in.) filter paper which was hung vertically from a trough. The trough and filter paper sheets were then suspended inside a closed vessel whose atmosphere was kept saturated with respect to the solvent. The trough was filled with the solvent and sheets were irrigated at room temperature to within 2 in. of the bottom. The sheets were dried and sprayed with silver nitrate solution. The sprayed sheets were then dried.

The ethyl and methyl alcohols used as solvents were purified prior to use by refluxing with zinc dust in an alkaline solution for 60 min. before redistillation. Phenylhydrazones were prepared by allowing solutions to stand at room temperature overnight in contact with an equal volume of 10% phenylhydrazine hydrochloride. Ether extracts were prepared by adjusting solutions to pH 5.5 and extracting with ether for six hours in a continuous extractor. The methods used in Warburg work are those described in previous papers (1, 7).

Experimental

Although the only information available on the use of paper chromatography for the study of intermediate carbohydrate metabolism was a preliminary report by Forsyth and Webley (3) on the hydrolysis of sucrose to the constituent hexoses by *Leuconostoc mesenteroides*, exploratory studies with *Pseudomonas aeruginosa* indicated that the technique would be applicable to the compounds and the conditions used in the present study.

The following compounds were found to give a definite spot with the silver nitrate reagent: glucose, glucose-1-phosphate, glucose-6-phosphate, hexose diphosphate, gluconic acid, 2-ketogluconic acid, 5-ketogluconic acid, α-ketoglutaric acid, pyruvic acid, lactic acid, malic acid, citric acid, glycerol, glycerol phosphate, and fructose. R_F values of these compounds are recorded in Table I. No reduction was given by succinic acid, fumaric acid, maleic acid,

TABLE I*

R_F VALUES OF KNOWN COMPOUNDS (2 MGM. PER ML.) IN 45% METHYL ALCOHOL, 45% ETHYL ALCOHOL, AND 10% WATER

Compound	R_F	Remarks
Glucose	0.59	
Glucose-1-phosphate	0.72	
Glucose-6-phosphate	0.36	
Hexose diphosphate	0.03	
Gluconic acid	0.425	
2-Ketogluconic acid	0.45	
5-Ketogluconic acid	0.45 0.03	This compound always gave two spots. It was not considered a possible intermediate since the organism could not grow with this compound as carbon source
α-Ketoglutaric acid	0.46	
Pyruvic acid	0.70	
Lactic acid	0.65	Yellow spot
Glycerol	0.76	
Malic acid	0.60	Orange-brown spot
Fructose	0.63	Grayish-brown spot
Citric acid	0.28	Orange spot
Glycerol phosphate	0.51	Faint orange spot

* *We are indebted to Dr. L. B. Lockwood of the Northern Regional Research Laboratory for samples of 2-ketogluconic and 5-ketogluconic acids; to Dr. C. E. Georgi of the University of Nebraska for a sample of 5-ketogluconic acid; to Dr. D. E. Green of the University of Wisconsin for a sample of α-ketoglutaric acid and to Dr. I. C. Gunsalus of the University of Indiana for samples of glucose-1-phosphate, glucose-6-phosphate, and hexose diphosphate.*

malonic acid, acetic acid, formic acid, formaldehyde, or acetone dicarboxylate. The spots obtained with silver nitrate were not always due to the presence of metallic silver; for instance, lactic acid gave a yellowish spot while citrate gave an orange spot. The gluconic acid spot was initially a yellowish shade turning dark brown with age. Although the silver nitrate reaction was not specific, many of these compounds could, however, be identified by color and R_F values.

Analysis of the culture supernatants always revealed at least two spots. One corresponded to glucose and the second with a smaller R_F value to some glucose degradation product. From a comparison of the R_F values obtained for known compounds, it was found that the value for this lower spot corresponded to that for gluconic, 2-ketogluconic, or α-ketoglutaric acid. Since the movement of these three acids was very similar in all the 55 combinations of solvents used, some supplementary means of separation had to be found.

Advantage was taken of the fact that 2-ketogluconic and α-ketoglutaric acids react with keto fixatives, whereas gluconic acid does not. The influence of sodium bisulphite, phenylhydrazine hydrochloride, hydroxylamine hydrochloride, semicarbazide, and 2 : 4-dinitrophenylhydrazine on the movement of these compounds was determined. Phenylhydrazine hydrochloride was

selected as the fixative to be used in further work, since the R_F values of the derivatives that were formed with it differed most markedly from those of the free acids while the R_F value of gluconic acid remained unchanged. An equal volume of a 10% solution of phenylhydrazine hydrochloride was allowed to react for 24 hr. at room temperature with the solution to be tested. These conditions permitted the complete conversion of any reasonable amount of 2-ketogluconate or α-ketoglutarate to its corresponding hydrazone.

The R_F values obtained, both before and after phenylhydrazine treatment, for known compounds and for the concentrated supernatant of a 24 hr. culture are recorded in Table II.

TABLE II

MOVEMENT OF INTERMEDIATE COMPOUNDS WITH 45% METHYL ALCOHOL, 45% ETHYL ALCOHOL, AND 10% WATER

	Before phenylhydrazine treatment	After phenylhydrazine treatment
(1) Glucose	0.55	0.66
(2) Gluconate	0.43	0.43
(3) 2-Ketogluconate	0.47	No spot
(4) α-Ketoglutarate	0.46	No spot
(5) Conc. uninoculated medium	0.55	0.66
(6) Conc. 24-hr. culture supernatant	0.45	0.43
(7) 1 : 1 dilution of (6)	0.44 0.48	0.46 No spot

In Tables II and III, the R_F values of spots corresponding to glucose in the supernatant and also those due to free phenylhydrazine have been omitted in order to minimize confusion. It can be clearly seen from these results (Table II) that the supernatant contained gluconic acid since a reducing spot with an R_F value corresponding to this compound remained after phenylhydrazine treatment. However, in each case the size of the spot was considerably reduced by phenylhydrazine treatment, indicating that some compound that would react with phenylhydrazine had been removed. On dilution of the concentrated supernatant with an equal volume of water, the larger single spot, which had an R_F of 0.45, was seen to actually be composed of two smaller spots, one with an R_F of 0.44 and the other with an R_F of 0.48. The spot with an R_F of 0.44 was gluconic acid since it remained undiminished after phenylhydrazine treatment, while the spot with an R_F of 0.48 could be either 2-ketogluconic or α-ketoglutaric acid or a mixture of these two compounds since it was eliminated by phenylhydrazine treatment.

In order to confirm the findings recorded in Table II, a second solvent containing 90% redistilled ethyl alcohol and 10% water was used (Table III).

TABLE III

MOVEMENT OF INTERMEDIATE COMPOUNDS WITH 90% ETHYL ALCOHOL AND 10% WATER

	R_F values	
	Before phenylhydrazine treatment	After phenylhydrazine treatment
(1) Glucose	0.56	0.56
(2) Gluconate	0.33	0.33
(3) 2-Ketogluconate	0.33	No spot
(4) α-Ketoglutarate	0.32	No spot
(5) Conc. uninoculated medium	0.56	0.54
(6) Conc. 24-hr. culture supernatant	0.36	0.35
(7) Conc. 16-hr. culture supernatant	0.41	0.36

In this experiment, the concentrated supernatant from a 16 hr. culture was analyzed with a view to demonstrating the presence of the same intermediate compounds in young cultures. The lower reducing spot observed previously was again detected and it remained after treatment with phenylhydrazine but was reduced in size. These findings indicate the presence of gluconic acid and a keto acid in the 16 hr. supernatant as well as in the 24 hr. supernatant.

Further separation of reducing compounds was attempted on the basis of the relative solubilities of α-ketoglutaric acid, 2-ketogluconic, and gluconic acids in ethyl ether. Gluconic acid, 2-ketogluconic acid, α-ketoglutaric acid, concentrated uninoculated medium, concentrated 24 hr. supernatant, and concentrated 16 hr. supernatant were ether extracted for six hours at pH 5.5. The ether soluble and ether insoluble fractions of all solutions as well as fractions of each solution that had not been extracted were analyzed using a solvent consisting of 95% ethyl alcohol and 5% ammonium hydroxide.

TABLE IV

R_F VALUES OF ETHER FRACTIONS OF CULTURE CONSTITUENTS

Compound	R_F	R_F ether insol. residue	R_F ether sol. fraction
Glucose	0.42		
Gluconate	0.15	0.15	No spot
2-Ketogluconate	0.143	0.142	No spot
α-Ketoglutarate	0.056	No spot	0.06
Medium	0.40	0.38	No spot
24-Hr. supernatant	0.39	0.40	No spot
	0.135	0.142	
16-Hr. supernatant	0.39	0.42	No spot
	0.135	0.152	

The ether fractions were treated with 10% phenylhydrazine hydrochloride with results similar to those recorded in Tables II and III. Again the reducing

spot representing the intermediate products was reduced but not completely removed by the phenylhydrazine treatment, indicating that gluconic acid and 2-ketogluconic or α-ketoglutaric acids or both were formed by the organism. Since no product could be detected in the ether extract of the culture, it can be concluded that α-ketoglutarate was not present in detectable quantities, and that 2-ketogluconic acid was the keto acid present.

Further confirmation of the presence of gluconic acid in the culture supernatants was recorded in every experiment. Shortly after the chromatogram was sprayed with silver nitrate, gluconic acid came up as a characteristic yellow spot which later turned brown and a spot with the same characteristics and with the same R_F value always appeared in the culture supernatant.

Curves showing the ability of *P. aeruginosa* to oxidize gluconic and 2-ketogluconic acids are presented in Fig. 1. These data combined with the chromatographic analysis of the 16 and 24 hr. cultures indicate that gluconic and 2-ketogluconic acids are being formed and oxidized continually and that they are not just the end products of a minor side reaction.

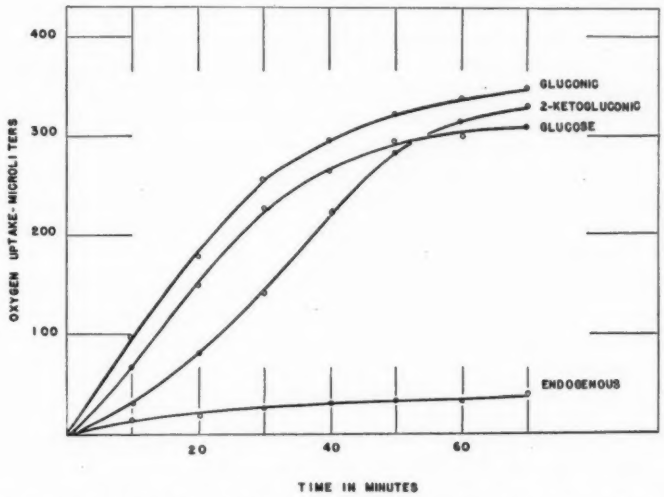

FIG. 1. *Oxidation of glucose, gluconic, and 2-ketogluconic acids by 22-hr. cells harvested from glucose ammonium phosphate medium.*

Warburg cups contained 0.5 ml. cell suspension, 1.5 ml. M/15 phosphate buffer, pH 7.2, 0.2 ml. substrate. Final volume was 3.0 ml. Theoretical oxygen uptake for complete oxidation of any substrate was 403 µl.

Discussion

The use of paper chromatography for the detection of gluconic, 2-ketogluconic, or α-ketoglutaric acids had not been previously reported and although a satisfactory procedure was eventually evolved, numerous difficulties were

encountered and a great deal of background information had to be accumulated. In searching for a solvent for the chromatographic analysis, one that would not interfere with the silver reduction reaction, would move the compounds being tested, and would be readily volatilized was essential. Saturated phenol, collidine, and butanol – acetic acid, the solvents commonly employed for the chromatographic analysis of reducing sugars (8), were found to be unsuitable as solvents since they did not move the possible degradation products of glucose through a sufficient distance to allow differentiation. It was found that methyl and ethyl alcohols at various concentrations, alone or in combination, served as excellent solvents for the compounds being tested. The alcohols were easily purified and they had the additional advantage of being volatile and thus readily removed.

The R_F values obtained were found to vary quite markedly with small differences in the water content of the solvent. It was therefore necessary to run known solutions with each individual chromatogram rather than use values obtained in previous determinations. Salts of acids or free acids were always found to give identical R_F values.

From the data presented it can be concluded that both gluconic and 2-ketogluconic acids were present in detectable amounts in a 16 hr. and 24 hr. culture of *P. aeruginosa* grown in glucose ammonium phosphate liquid medium. It was also found that in a 38 hr. culture all the glucose had disappeared and only the faintest trace of gluconic or 2-ketogluconic acid could be detected chromatographically. It has also been shown that *P. aeruginosa* has a strong mechanism for oxidizing both of these compounds. One must therefore conclude that these compounds are being formed at a continuous and vigorous rate and are being removed in the same manner. This can only mean that these compounds are part of the system through which most if not all of the glucose is oxidized.

The fact that cells harvested from a glucose medium require a period of adaptation before oxidizing 2-ketogluconic acid confirms the earlier evidence (1) that the technique of simultaneous adaptation is not applicable to studies such as these.

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X-RAY TOLERANCE OF LIVING CELLS AS MEASURED BY CYTOPLASMIC STREAMING[1]

By Charles J. Bishop,[2] Victor D. McLaughlin,[3] and Donald F. Tapley[3]

Abstract

An investigation was undertaken to determine the immediate effects of X radiation on living, nondividing cells using the rate of cytoplasmic streaming as a measure of cell vitality. The observations were carried out on stamen hairs and pollen tubes of *Tradescantia paludosa* (diploid) and *Tradescantia virginiana* (tetraploid). No significant variation in the rate of streaming of cytoplasm in either stamen hairs or pollen tubes was noted with low doses of X rays. In both diploid and tetraploid stamen hairs, 700,000 r. was required to stop all streaming. In the pollen tubes of diploid and tetraploid plants, all streaming was stopped by 250,000 r. and 200,000 r. respectively, a difference that was statistically significant. Greater sensitivity, as shown by resulting cell death, was found with an increased initial dosage of continuous irradiation.

Introduction

The vitality of certain living plant cells may be determined by observing the rate of cytoplasmic streaming. In cells where this normally is visible, a decrease in the rate of streaming is considered indicative of lowered vitality, and a complete cessation is generally assumed to indicate death, accompanied by the ending of all normal cell functions.

The present investigation was undertaken to determine the immediate effects of X radiation on living, nondividing cells, using the rate of cytoplasmic streaming as an indication of cell vitality.

As early as 1898 small doses of X radiation were observed by Lopriore (4) to produce an acceleration in the movement in protoplasm. Similar results were noted by Williams (11) and Nadson and Rochlin (5), who, in addition, found that the period of acceleration was soon followed by signs of depression. Wanner (9) using root hairs of *Hydrocharis morsus ranae* and epidermal cells of *Allium Cepa* found that, after irradiation with 400 to 10,000 roentgens,* there was an immediate decrease in viscosity of the protoplasm in epidermal cells of *Allium* and an increase in the rate of streaming in the root hairs of *Hydrocharis*. He found that the maximum speed increase in its absolute and relative magnitude showed no distinct dependence on the radiation dose, and that up to 6,000 r., the original speed recurred after a 10 hr. period of acceleration. Wanner also stated that, from 6000 to 12,000 r., permanent damage to the protoplasm and the ultimate death of the cell followed the period of acceleration. With doses exceeding 12,000 r., an immediate depression of

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* *A roentgen is the unit of measurement of radiation dosage established by international agreement for all scientific research.*

the rate of streaming, followed by damage to the protoplasm, was noted. The dosage in Wanner's experiments was not increased to the point at which protoplasmic streaming stopped immediately, but rather an initial dose was administered and changes observed in viscosity (by centrifuging) or in the rate of streaming (by observation) for periods as great as 50 hr. The present investigations were carried to the point where sufficient X rays were administered to cause an immediate cessation of cytoplasmic streaming and thus, presumably, death of the cell.

Materials and Methods

Cytoplasmic streaming was observed in the stamen hairs and pollen tubes of *Tradescantia paludosa*, a diploid species, and in *Tradescantia virginiana*, a tetraploid species. The rate of streaming, in microns per minute, was determined by observing the flow of cytoplasm, using a calibrated ocular micrometer. By this means a comparison was made between the radiation sensitivities of the diploid and tetraploid species.

The stamen hairs were cultured on an agar medium of 7.5% sucrose, 1% agar in distilled water. In both cases the medium was applied to a cover glass inverted over a Van Tieghem cell, which was used as a modified moist chamber (2). This technique made it possible to subject the material to long periods of irradiation without danger of the culture drying, and allowed observation of the cells with both low and high power objectives.

In each experiment cultures were first checked for rates of streaming and then subjected to a large uninterrupted dosage of radiation, followed by dosages of 25,000 r. each, until cytoplasmic streaming ceased. Observations were taken between each period of irradiation. For stamen hairs the initial dosage was 250,000 to 450,000 r., and for pollen tubes was 100,000 to 125,000 r.

The X radiation was from a standard Coolidge type X-ray tube having an inherent filtration of 1 mm. of aluminum and operated at 140 kv. and 5 ma. with a beam intensity of 1200 r.p.m. Calibrations were made with a Victoreen dosimeter, duplicating as nearly as possible the conditions under which the material was irradiated. In doing this, the ionization chamber of the dosimeter was placed above a microscope slide, surrounded on three sides by a glass arc, and covered by a cover glass of the same thickness used in the experiments.

Results

In stamen hairs, no significant variation in the rate of cytoplasmic streaming was observed with low doses of radiation. In pollen tubes, the normal variation within a single cell was too great to permit the observation of a change in speed as small as that to be expected with the low doses.

Large doses of X rays invariably caused a cessation of cytoplasmic streaming both in stamen hairs and in pollen tubes. The effect of the X rays was found in all cases to be permanent. With pollen tubes, this is less significant because streaming normally stops after approximately 15 hr. On the other hand,

stamen hairs in which the rate of streaming had been reduced to 150 μ per minute by irradiation remained unchanged for 96 hr. In another experiment, cells where activity had been reduced to Brownian movement were observed to be in the same condition at the end of 24 hr.

The results of experiments conducted on stamen hairs are presented graphically in Fig. 1. As indicated by the curves, the streaming became progressively slower up to 650,000 r. Thereafter, there was either no streaming at all or

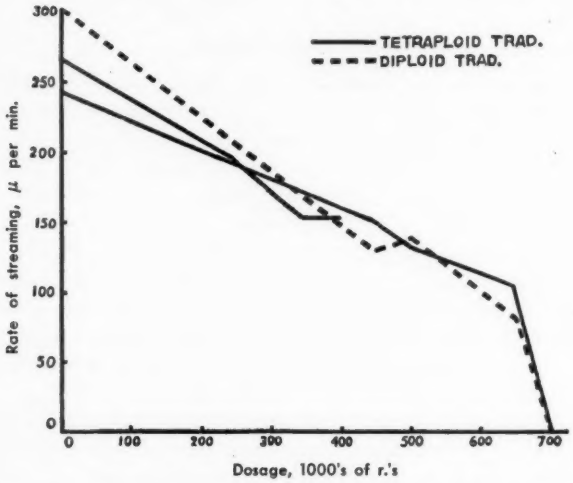

FIG. 1. *The effect of X rays on cytoplasmic streaming in stamen hairs of diploid and tetraploid Tradescantia.*

the streams were too short to permit accurate timing. Brownian movement was evident up to 750,000 r., but was not recorded as streaming. In the two experiments that were taken to completion, the initial dose as indicated by the graph was 450,000 r., whereas in the other experiment shown, the initial dose was 250,000 r. As shown by the graph, no significant difference was found between diploid and tetraploid stamen hairs.

In the case of pollen tubes, in which streaming normally stops after 15 to 20 hr. of growth, X rays caused a much earlier cessation of cytoplasmic streaming. There was no significant increase in the percentage bursting of the tubes, and their appearance was more normal than that of tubes dying a natural death, which usually have a granular appearance. There was no thickening of the walls of the pollen tubes as is frequently the case when death is normal.

The effect of X rays on cytoplasmic streaming in the pollen tubes of diploid and tetraploid plants is indicated in Fig. 2. This graph represents the average results of all the experiments conducted on pollen tubes. In none of the

experiments included in this graph were readings taken before 100,000 r. had been administered. From 100,000 to 250,000 r., readings were taken at intervals of 25,000 r., and each point on this graph has been determined by averaging approximately 45 readings. At each interval, a lapse of 10 min.

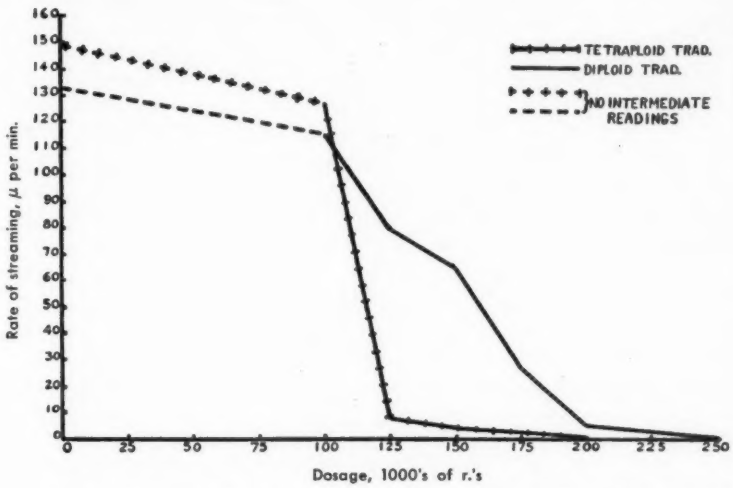

FIG. 2. *The effect of X rays on cytoplasmic streaming in pollen tubes of diploid and tetraploid Tradescantias.*

between irradiations was required to make accurate observations. A continuous irradiation of 125,000 r., administered to some of the pollen tubes of tetraploid Tradescantias frequently caused a complete cessation of streaming. This considerably lowered the average rate of streaming at this point for the whole series, and partially explains the sudden drop in the tetraploid curve between 100,000 and 125,000 r.

As is indicated by the graph, the maximum lethal dosage for the pollen tubes of tetraploid Tradescantias was 200,000 r., while for diploid plants it was 250,000 r. The average end point for tetraploid plants was 154,625 ± 10,500 r., and for the diploids 208,500 ± 15,700 r. A statistical analysis of the data shows that this difference is highly significant, indicating that in our experiments the diploid cells were more resistant than the tetraploid ones.

Fig. 3 is a graphical representation of one of the experiments included in the graph of Fig. 2. At each point where a reading is noted, there was a lapse of 10 min. between irradiations. In this experiment, all four cultures were made with the same medium and pollen from a single flower of each species was used. The graph indicates the difference between the pollen tubes of diploid and tetraploid Tradescantias and also shows the variation produced by changing the initial dose. Tubes from both the diploid and tetraploid

species given an initial dose of 100,000 r. required a greater amount of irradiation to stop the streaming than did those given an initial dose of 125,000 r. Thus it is shown that fractionation reduces the effectiveness of a given dose.

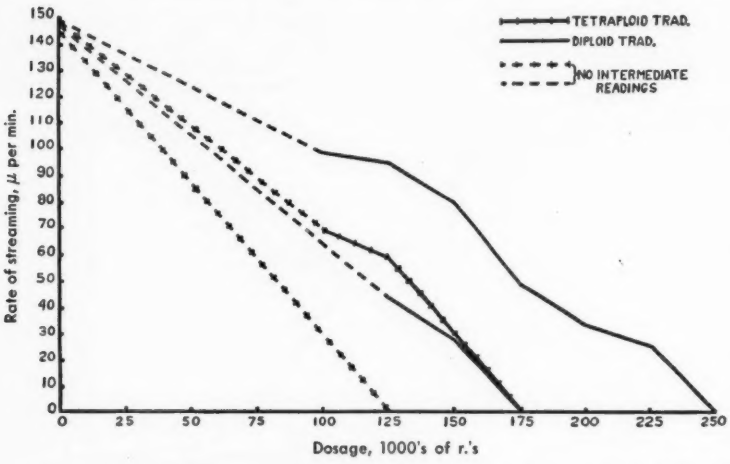

FIG. 3. *The effect of varying the initial dose on cytoplasmic streaming in pollen tubes of diploid and tetraploid Tradescantias.*

Discussion

With few exceptions, experiments on the effects of irradiation on cells have been concerned with cell reproduction, and particularly in recent years there have been few studies based on the immediate reaction of cells to X rays. Many of the early experiments preceded the use of accurate means of measuring the amount of irradiation, and dosages were expressed mainly as a function of time and distance.

In experiments on *Paramecium* testing the immediate effects of large doses of X rays, Back and Halberstaedter (1) and Wichterman (10) have shown that these animals could survive doses up to 600,000 and 700,000 r. The recent work by Wanner (9) indicates clearly a similar tolerance of cells of *Allium* and *Hydrocharis* to X radiation. The latter work was, like our own, based to a considerable extent on the rate of cytoplasmic streaming. In Wanner's experiments no dosages were used exceeding 48,000 r., and these were not immediately lethal to the cells, although they did cause death in periods up to 50 hr. following treatment.

No significant increase in the rate of streaming was observed in any of our experiments using dosages less than 10,000 r. This is perhaps merely an indication of the great variation that exists in protoplasm. Thus, Back and Halberstaedter (1) found no visible effects on *Paramecia* motility from doses of less than 100,000 r., Wichterman (10) showed increased motility with

100,000 r., Northen and MacVicar (6) found a decrease in protoplasmic viscosity in *Spirogyra* cells, with doses of 250 to 5000 r., and Wanner (9) showed that in *Allium* and *Hydrocharis* both a decrease in viscosity and an increase of about 15% in the rate of streaming followed irradiation up to 12,000 r. Nadson and Rochlin (5) reported an acceleration of cytoplasmic movement in yeast as one of the first effects of irradiation, but they made no accurate measurement of doses used. In our cultures, variations within a single cell, particularly in the case of pollen tubes, may have been sufficient to mask any minor changes. In any case, none were observed.

Though reported by many investigators (Rochlin-Gleichgewicht (7), Williams (11 and 12), and Heilbrunn and Mazia (3)), a conspicuous change in vacuolization was not seen following irradiation of the living cells in the present experiments. Even those that were killed by the radiation seemed more homogeneous than cells that died a natural death.

With stamen hairs (Fig. 1), our data show a continuous decrease within the limits of the points of observation until the streaming ceased completely and the cell died. The extreme resistance of mature stamen hairs, which are in a permanent resting stage, to large doses (up to 750,000 r.) of X rays is of particular interest. It appears even more striking when one considers that in certain sensitive stages where cell reproduction is involved 200 r. will cause as much as 50% pollen sterility in *Tradescantia* (8).

Pollen tubes were found to be considerably more sensitive than the stamen hairs and all were "killed" by dosages of 250,000 r. or less. They are much more active than stamen hairs and represent cells in the early prophase stage. This would seem to indicate that the sensitivity of the cell may be related to the stage of the cell nucleus, but with the material used this was impossible to check.

Continuous irradiation has a more detrimental effect on the rate of cytoplasmic streaming than does irradiation administered with frequent rest periods. This is clearly illustrated in Fig. 3, where both diploid and tetraploid tubes, given an initial dose of 100,000 r., required a greater amount of irradiation to stop the streaming than did those given an initial dose of 125,000 r. The brief rest period apparently allowed some sort of recovery to take place within the cell.

Polyploidy caused no difference in effect in the case of stamen hairs, but with the pollen tubes a statistical analysis indicated a significant difference between the effects on the pollen tube of the diploid and tetraploid species, the latter showing greater sensitivity. This, of course, may represent simply a species difference rather than a change caused by an increase in chromosome number, for the two species naturally would possess genetical differences.

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